MICROBIOLOGICAL REVIEWS, Sept. 1979, p. 384-421
0146-0749/79/03-0384/38$02.00/00

Vol. 43, No. 3

Bacterial Iron-Sulfur Proteins

DUANE C. YOCH[1]* AND ROBERT P. CARITHERS[2]

Department of Biology, University of South Carolina, Columbia, South Carolina 29208,[1] and Department of Biochemistry, Rice University, Houston, Texas 77001[2]

INTRODUCTION

Iron-sulfur proteins constitute a group of electron transport proteins (ferredoxins) and oxidation-reduction enzymes whose function is now known to be central to such important cellular processes as photosynthesis, nitrogen fixation, respiration, and carbon dioxide fixation. The relationship between the ferredoxin electron carriers and iron-sulfur-containing oxidoreductases may be thought of as analogous to that between cytochromes and heme-containing enzymes, respectively. The ferredoxins differ from their cytochrome counterparts in that they generally function at negative oxidation-reduction potentials (−340 to −480 mV), although in recent years iron-sulfur centers in some enzymes have been determined to be as positive as +50 mV, and the high-potential iron protein (HiPIP), a ferredoxin of unknown function, has a redox potential of approximately +350 mV.

The distinguishing feature of iron-sulfur proteins is the prosthetic group, which contains iron, commonly referred to as nonheme iron (16), bound to the peptide chain through four cysteinyl-sulfur ligands. The simplest group of

nonheme iron proteins, the rubredoxins, contain a single iron atom bound to the peptide by four cysteinyl-sulfur bonds. Because rubredoxins lack inorganic sulfur, a characteristic common to the vast majority of iron-sulfur proteins, they are not discussed here (see Lovenberg [162] for a review of this topic). The classical nonheme iron proteins contain inorganic or "acid-labile" sulfur (designated S*) in addition to the nonheme iron atoms. The term acid-labile as a description for the sulfur atoms in the prosthetic group recognizes that the sulfur has a −2 valence and that acid treatment liberates it as H_2S during denaturation of the protein. The current concept concerning the iron-sulfur protein prosthetic group is that it is limited to clusters of either two irons and two sulfides (Fe_2S_2*) or clusters of four irons and four sulfides (Fe_4S_4*) (Fig. 1). Any individual protein would therefore contain one or more copies of these basic Fe-S structures. In a number of iron-sulfur enzymes, one or more of these clusters function in conjunction with heme, flavin, pteridine, or molybdenum cofactors or with a combination of these cofactors. In the molybdenum-containing enzymes there is the possibility that an iron-sulfur-molybdenum center may also exist and play an important role in nitrogenase (215), whereas the evidence for similar centers in xanthine oxidase and certain nitrate reductases is less convincing.

In the 15 years since *Bacteriological Reviews* published what appears to be the first review article on iron-sulfur proteins (290), over 100 iron-sulfur proteins have been isolated from various plant, mammalian, and bacterial sources and studied in varying detail, resulting in over 1,000 research publications. Because of this enormous literature, this review focuses only on the bacterial iron-sulfur proteins, and furthermore, it is restricted to the more recent developments in this field. It is our goal to discuss the physical and chemical nature of the bacterial iron-sulfur clusters as they are found in enzymes and ferredoxins and to relate this information to the general physiology of the cell. More detailed information on individual iron-sulfur proteins and the function of these proteins, including those from plant and animal sources, may be found in recent books edited by Lovenberg (163), the definitive source, and Neilands (194) and in review articles (170, 223).

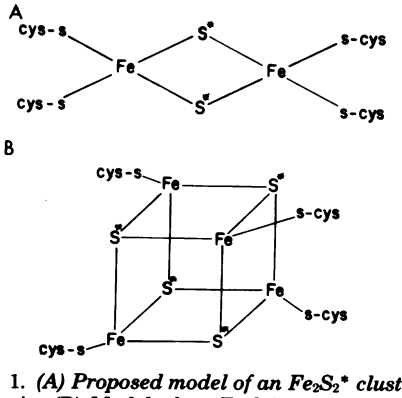


FIG. 1. *(A) Proposed model of an Fe_2S_2* cluster of ferredoxin. (B) Model of an Fe_4S_4* cluster of a bacterial ferredoxin derived from X-ray diffraction studies.*

FERREDOXINS

Properties of Fe_2S_2* Ferredoxins

Although it was thought for some time that the 2Fe-2S* ferredoxins were restricted to plant chloroplasts (266) and blue-green algae (cyanobacteria) (249) (they are still referred to as "plant-type" ferredoxins), they have also been found in bacteria (Table 1); however, they are relatively rare compared to those with tetranuclear iron clusters (see Table 2).

The structure of the Fe_2S_2* cluster (Fig. 1A) was originally derived indirectly from a large body of magnetic and spectroscopic data (39, 77, 283), and a recent X-ray crystallographic analysis of a two-iron ferredoxin from *Spirulina platensis* has confirmed this structure (287). The iron atoms of the Fe_2S_2* cluster are bound to the polypeptide chain by four cysteinyl-sulfur residues and to each other by two bridging labile sulfur ligands. The iron atoms are nearly tetrahedral in their coordination to the four sulfur atoms.

Iron-sulfur compounds of the general formulation $[Fe_2S_2(SR)_4]^{2-}$ have been synthesized and crystallized, and X-ray analyses of these crystals have shown their structure to be nearly identical to that of the 2Fe-2S cluster shown in Fig. 1 (173). Furthermore, the observation that the Fe_2S_2* center from the ferredoxin of *Spirulina maxima* (a cyanobacterium) can undergo thiolate substitution reactions to form a product indistinguishable from the synthetic analog (219) provides additional evidence that the Fe_2S_2* centers of the two-iron ferredoxin and the synthesized cluster are identical. In the extrusion reaction (121) thiolate groups, RS—, substitute for the cysteinyl sulfur which binds the Fe-S group to the peptide, allowing the iron-sulfur core (either Fe_2S_2* or Fe_4S_4*) to be removed intact from the peptide. This reaction is shown by the equation:

$$[Fe_2S_2^*(SR)_4]^{2-} + 4R'SH \rightleftharpoons [Fe_2S_2^*(SR')_4]^{2-} + 4RSH$$

where R'SH represents thiol.

TABLE 1. *Properties of bacterial Fe_2S_2* ferredoxins*

Compound	Mol wt	Redox potential (E_m) (mV)	EPR properties		Biological function	Reference(s)
			g values	Symmetry		
Pseudomonas putida ferredoxin	12,000	−235	1.94, 2.01	Axial	Camphor hydroxylation	63, 282
Halobacterium halobium ferredoxin	14,800	−345	1.90, 1.97, 2.07	Rhombic	α-Keto acid oxidation	142
Escherichia coli ferredoxin	12,600	−380	1.94, 2.02	Axial	Unknown	147, 296
Agrobacterium tumefaciens ferredoxin	ND[a]	−223	1.94, 2.02	Axial	Unknown	293
Clostridium pasteurianum paramagnetic protein	25,000	−300	1.94, 1.96, 2.0	Rhombic	Pyruvate oxidation	52, 115
Azotobacter vinelandii Fe-S protein I[b]	21,000	−350	1.92, 1.94, 2.02	Rhombic	Unknown	72, 157, 240
Azotobacter vinelandii Fe-S protein II	21,000	−225	1.91, 1.93, 2.03	Rhombic	Protection of nitrogenase from O_2	21, 157, 233, 240
Nostoc strain MAC ferredoxin I	12,050	−350	1.92, 1.96, 2.06	Rhombic	Photosynthesis, N_2 and NO_2^- reduction	49, 127, 128
Nostoc strain MAC ferredoxin II	12,250	−455	ND	ND	ND	49, 127, 128

[a] ND, Not determined.
[b] Not to be confused with *A. vinelandii* ferredoxin I (Table 2).

Electron paramagnetic resonance (EPR) spectroscopy, which reveals paramagnetic centers, shows that the reduced Fe_2S_2* center has a prominent signal at $g = 1.94$, as shown in Fig. 2 by the two-iron center of the spinach ferredoxin. (An exception to this is the Rieske protein [225], which is discussed in detail below.) Evidence suggests that the Fe_2S_2* cluster of a ferredoxin has only two oxidation states. Mössbauer spectroscopy data indicate that both iron atoms are in the ferric state in the oxidized cluster and that, on reduction of the cluster by a single electron, one of these iron atoms is reduced to the ferrous state (77, 102) and the iron-sulfur cluster becomes paramagnetic due to the unpaired electron on the ferric atom. The EPR data, along with the data derived from studies of the 2Fe-2S analogs, indicate that the Fe_2S_2* cluster in both ferredoxins and iron-sulfur enzymes functions between the $(Fe_2S_2{}^*S_4{}^{cys})^{2-}$ and $(Fe_2S_2{}^*S_4{}^{cys})^{3-}$ valence states. The midpoint potential (E_m) of this transition varies from one ferredoxin to another, but it is in the range of −250 to −400 mV (Table 1); in iron-sulfur enzymes the E_m's of the Fe_2S_2* cluster appear to be more positive. In addition to the changes noted in the magnetic properties of the Fe_2S_2* cluster, chemical reduction of this center is also accompanied by a decrease of about 50% in the visible absorbance bands located at approximately 330, 420, and 460 nm.

Reconstitution of a typical 2Fe-2S* protein (*Pseudomonas putida* ferredoxin) with either ^{57}Fe or ^{33}S resulted in line broadening of the EPR signal and provided strong evidence for the involvement of both the iron and the sulfide in the electron-accepting center; a similar observation was made when either the labile sulfur or cysteinyl sulfur was chemically exchanged with ^{33}S (282; unpublished data cited in reference 199). The strict conservation of the cysteine positions in the amino acid sequence of the Fe_2S_2* ferredoxins from plants and cyanobacteria further suggests involvement of cysteinyl sulfur in the active center (113, 309).

The molecular weights of the two-iron ferredoxins from plants and cyanobacteria are, without exception, about 12,000, but the molecular weights of the two-iron ferredoxins from bacteria fall into two groups, one 12,000 and the other approximately 24,000. The significance of this difference in molecular weights is not yet understood, but it may in some way be related to the biochemical function of these proteins. The 12,000-dalton ferredoxins from *P. putida* and *Halobacterium halobium* play conventional electron transport roles in such reactions as camphor hydroxylation (136) and α-keto acid oxidation (141), whereas one of the 24,000-dalton Fe_2S_2* proteins, *Azotobacter* iron-sulfur protein II (72), is reported to play a role in protecting the nitrogenase from O_2 inactivation (233). The function of the other two-iron ferredoxins, such as those from *Escherichia coli* (296), *Clostridium pasteurianum* (115), and *Agrobacterium tumefaciens* (293), is unknown.

Properties of Fe_4S_4* Ferredoxins

The structure of the Fe_4S_4* cluster has been determined by X-ray crystallographic studies of two bacterial iron-sulfur proteins, the 4Fe-4S* HiPIP from *Chromatium vinosum* (54) and the 8Fe-8S* ferredoxin from *Peptococcus aerogenes* (246) (the latter of which contains two Fe_4S_4*

clusters). The Fe_4S_4* centers from these two bacterial electron carrier proteins were found to be nearly identical (Fig. 1B). As Fig. 1B shows, the Fe_4S_4* cluster has sulfide bridges between each of the four iron atoms, and the iron in this cluster is bound to the protein skeleton via four cysteinyl sulfurs. It is assumed that the Fe_4S_4* centers of other ferredoxins and the iron-sulfur-containing enzymes have a similar structure because of their similarity in optical and magnetic properties.

The Fe_4S_4* clusters are thought to have three possible oxidation states in biological systems (the three-state hypothesis) (55, 118, 121). In its most oxidized state, $(Fe_4S_4{*}S_4^{cys})^{1-}$, the center is paramagnetic and is representative of the oxidized HiPIP center; it exhibits a relatively featureless symmetric EPR spectrum with a resonance peak near $g = 2.01$ (Fig. 2). A one-electron reduction of the most oxidized form of the cluster results in $(Fe_4S_4{*}S_4^{cys})^{2-}$, which is diamagnetic and therefore EPR silent; this center is indicative of the reduced HiPIP and oxidized clostridial-type ferredoxins. A further one-electron reduction generates $(Fe_4S_4{*}S_4^{cys})^{3-}$, which is paramagnetic and representative of reduced clostridial-type ferredoxins; this center has a major resonance peak near $g = 1.94$ (see EPR spectrum of *Bacillus stearothermophilus* ferredoxin [Fig. 2]). Evidence in support of the three-state hypothesis is as follows: (i) the reduced $(Fe_4S_4{*}S_4^{cys})^{2-}$ diamagnetic cluster of *Chromatium* HiPIP could be further reduced to a paramagnetic $(Fe_4S_4{*}S_4^{cys})^{3-}$ state with the typical $g = 1.94$ EPR spectrum (48); and (ii) Sweeney et al. (264) observed that oxidation of the predominantly diamagnetic $(Fe_4S_4{*}S_4^{cys})^{2-}$ clusters of clostridial ferredoxin with ferricyanide produced a superoxidized iron-sulfur cluster with an EPR signal at $g = 2.01$, indicative of the -1 valence state. Both experiments (which involved unfolding of the Fe-S proteins in solvents containing dimethyl sulfoxide) clearly demonstrated that the tetranuclear Fe-S cluster can exist in three oxidation states.

When Carter et al. (55) first proposed that the Fe_4S_4* cluster might exist in three oxidation states, it was thought that the electronic transition between the -1 and -2 oxidation states occurred only at relatively high redox potentials, whereas the transition between -2 and -3 states occurred only at low potentials. Recent evidence from studies on succinate dehydrogenase (197), *Azotobacter vinelandii* ferredoxin I (265), and the *Desulfovibrio gigas* ferredoxins (50) indicates that one cannot predict from the operational valence states of the iron-sulfur cluster the potential range over which these oxidation-reduction transitions occur. This suggests that the protein moiety determines not only whether the iron-sulfur center cycles between the $-1 \leftrightharpoons -2$ or $-2 \leftrightharpoons -3$ oxidation states, but also determines the redox potential at which these transitions occur.

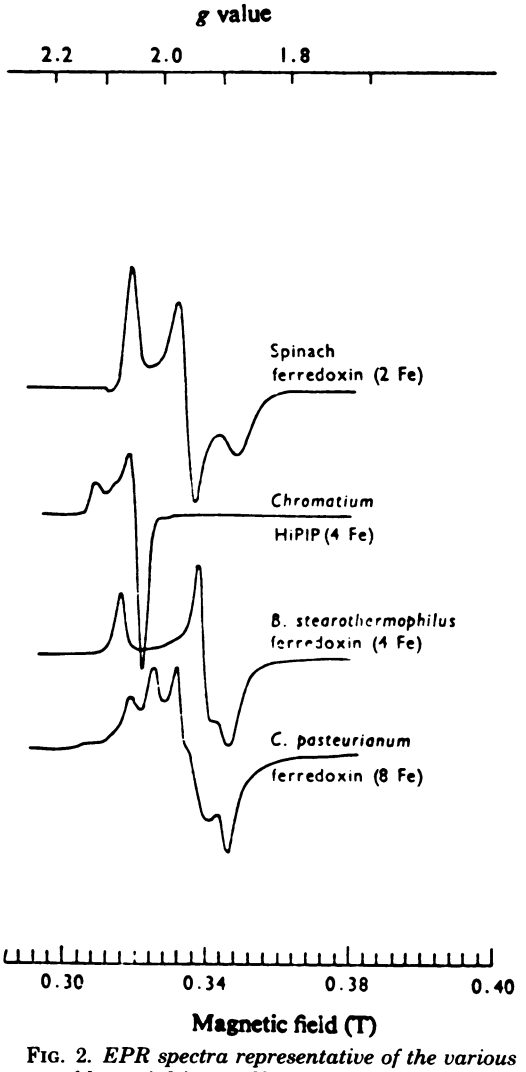


FIG. 2. *EPR spectra representative of the various types of bacterial iron-sulfur centers (modified from Hall et al. [113]). Chromatium HiPIP was in the oxidized state, and the other iron-sulfur proteins were in reduced state; all EPR measurements were at about 20°K.*

As Table 2 shows, the bacterial ferredoxins that contain Fe_4S_4* centers are found in a large number of microorganisms. In this table, the ferredoxins are divided into electron carriers with (i) a single Fe_4S_4* cluster, (ii) two Fe_4S_4* clusters that function at the same oxidation-reduction potential, and (iii) two or more Fe_4S_4*

TABLE 2. *Characteristics of ferredoxins which contain Fe_4S_4* clusters*

Source	Ferredoxin designation	No. of clusters	E_m (mV)	Redox transition[c]	Mol wt	Reference(s)
Ferredoxins with a single Fe_4S_4* cluster						
Desulfovibrio gigas (monomer)		1	ND[a]	$-2 \leftrightharpoons -3$	6,200	152
Bacillus stearothermophilus		1	−280	$-2 \leftrightharpoons -3$	8,000	188
Bacillus polymyxa	FdI	1	−390	$-2 \leftrightharpoons -3$	9,000	3, 202, 234, 261, 321
Bacillus polymyxa	FdII	1	−422	$-2 \leftrightharpoons -3$	9,000	261, 310
Clostridium thermoaceticum		1	ND	$-2 \leftrightharpoons -3$	7,300	308
Desulfovibrio desulfuricans		1	−330	$-2 \leftrightharpoons -3$	6,000	322
Mycobacterium flavum	FdII	1	ND	$-2 \leftrightharpoons -3$	ND	6
Rhodospirillum rubrum	FdII	1	−430	$-2 \leftrightharpoons -3$	14,500	239, 315
Spirochaeta aurantia		1	ND	$-2 \leftrightharpoons -3$	6,000	131
Chromatium vinosum	HiPIP	1	+350	$-1 \leftrightharpoons -2$	9,850	15, 54
Paracoccus sp.	HiPIP	1	+360	$-1 \leftrightharpoons -2$	ND	124, 269
Ferredoxins with two Fe_4S_4* clusters that function at the same E_m						
Chlorobium limicola	FdI & II	2	ND	$-2 \leftrightharpoons -3$	7,000	267, 268
Chromatium vinosum		2	−480	$-2 \leftrightharpoons -3$	10,000	12
Clostridium acidi-urici		2	−434	$-2 \leftrightharpoons -3$	6,000	45, 262
Clostridium pasteurianum		2	−390	$-2 \leftrightharpoons -3$	6,000	45, 206, 266
Peptococcus aerogenes		2	−427	$-2 \leftrightharpoons -3$	6,000	38, 262
Ferredoxins with two or more Fe_4S_4* clusters that function at different E_m's						
Rhodospirillum rubrum	FdI	2	ND	$-2 \leftrightharpoons -3$	14,000	239, 315
Azotobacter vinelandii	FdI	2	+320, −420	$-1 \leftrightharpoons -2$	14,500	265, 313, 318
Mycobacterium flavum	FdI	2	+230, −420[b]	$-1 \leftrightharpoons -2$	11,200	6
Rhodospirillum rubrum	FdIV	2	+355, −380	$-1 \leftrightharpoons -2$	14,000	319
Desulfovibrio gigas	FdI	3	−455	$-2 \leftrightharpoons -3 \gg -1 \leftrightharpoons -2$	18,000	40, 50, 152
Desulfovibrio gigas	FdI	3	−430, −30	$-2 \leftrightharpoons -3 \simeq -1 \leftrightharpoons -2$	18,000	40, 50
Desulfovibrio gigas	FdII	4	−130	$-2 \leftrightharpoons -3 \ll -1 \leftrightharpoons -2$	24,000	40, 50

[a] ND, not determined.
[b] M. G. Yates and H. Bothe, personal communication.
[c] See Addendum in Proof (p. 410).

clusters that operate at different redox potentials.

Ferredoxins with a single Fe_4S_4* cluster were first isolated from *D. gigas* (152) and *Bacillus polymyxa* (202, 241, 321). The occurrence of a single Fe_4S_4* cluster (instead of two Fe_2S_2* clusters) in four-iron ferredoxins was demonstrated by Stombaugh et al. (261), who showed that, on full reduction, *B. polymyxa* ferredoxin took up one electron per molecule (two electrons would be required to reduce a four-iron protein containing two Fe_2S_2* centers). These single-cluster ferredoxins generally have molecular weights between 6,000 and 10,000. From a comparison of the physical properties of these iron-sulfur proteins with those of model compounds, it was proposed that these ferredoxins cycle between the $(Fe_4S_4{}^*S_4{}^{cys})^{2-}$ and $(Fe_4S_4{}^*S_4{}^{cys})^{3-}$ oxidation states at potentials near −400 mV (118, 121).

The *Chromatium* HiPIP is an example of a class of four-iron electron carriers that cycles between the $-1 \leftrightharpoons -2$ oxidation states at a relatively high potential (+350 mV). Evidence for these redox transitions comes from the observation that oxidized HiPIP is paramagnetic (suggesting a −1 valence), whereas reduced HiPIP is diamagnetic (suggesting a −2 valence) in the reduced state (15). Taken with the model studies on $Fe_4S_4(SR)_4$ synthetic analogs (118, 121), the evidence for the $-1 \leftrightharpoons -2$ redox transitions in *Chromatium* HiPIP is quite compelling. X-ray crystallography data showing that the Fe_4S_4* cluster of *Chromatium* HiPIP (54) is strikingly similar to the Fe_4S_4* clusters of *P. aerogenes* ferredoxin (246) again suggest that the peptide determines both the oxidation state and the operational redox potential of the Fe_4S_4* cluster. In contrast to the vast amount of evidence that has been compiled on the physical and chemical characterization of *Chromatium* HiPIP, there is little or no evidence as to its biochemical role in the cell.

A number of ferredoxins normally referred to as clostridial-type ferredoxins have two Fe_4S_4* clusters which function at about the same negative redox potential. The two Fe_4S_4* clusters of *Clostridium acidi-urici* ferredoxin were shown, for example, to differ in potential by less than 10 mV (209). These ferredoxins are found primarily in anaerobic bacteria such as the clostridia and the photosynthetic bacteria; here they catalyze numerous two-electron transfer reactions, such

as hydrogen evolution and pyridine nucleotide reduction.

All of the known ferredoxins which function between the $-2 \leftrightharpoons -3$ oxidation states have molecular weights of 6,000 to 9,000. The complicated nature of the 8Fe-8S* *C. pasteurianium* ferredoxin EPR spectrum compared with that of the 4Fe-4S* *B. stearothermophilus* ferredoxin (Fig. 2) is believed to be a result of spin coupling between the two Fe_4S_4* clusters of the former (172).

The arrangement of the two Fe_4S_4* clusters in relation to the peptide in a clostridial-type ferredoxin has been determined by X-ray crystallography (3, 246) (Fig. 3). The protein backbone of the ferredoxin from *P. aerogenes* is folded in such a way that each of the Fe_4S_4* clusters, which are about 1.2 nm apart, is bound by cysteine residues from both ends of the polypeptide; that is, cysteines 8, 11, 14, and 45 are coordinated to iron atoms in one complex, and cysteines 18, 35, 38, and 41 are coordinated to the iron in the second complex (3). The position of the cysteinyl residues in ferredoxins from both fermenting and photosynthetic bacteria is rigorously conserved (309), suggesting a similar environment for the Fe_4S_4* clusters of these ferredoxins.

The ferredoxins with two iron-sulfur clusters that operate with different redox potentials belong to the third class of bacterial ferredoxins. These two-cluster ferredoxins are found in aerobes such as *A. vinelandii* (265) and *Mycobacterium flavum* (M. G. Yates and H. Bothe, personal communication) and are referred to here as *Azotobacter*-type ferredoxins. Unlike other 8Fe-8S* ferredoxins, these proteins exhibit a single EPR absorption band in the oxidized state at $g = 2.01$ (Fig. 4), which is now taken to be indicative of Fe_4S_4* centers which function between the $-1 \leftrightharpoons -2$ oxidation states, analogous to HiPIP. Electron spin quantitation by Sweeney et al. (265) indicated that only one of the two Fe_4S_4* centers was functional at low potential ($E_m = -420$ mV). When one electron was taken up, the $g = 2.01$ EPR signal of this low-potential center disappeared. The second Fe-S center of *Azotobacter* ferredoxin I was observed by optical and EPR spectroscopy only after oxidation of the protein with ferricyanide (265). This center, which is in the reduced state in the isolated protein, showed a positive E_m of +340 mV after equilibrium of the protein with the ferro-ferricyanide couple. This high-potential center, which was EPR silent (i.e., reduced) in the isolated protein, also had a $g = 2.01$ EPR signal, indicating that it also functions between the $-1 \leftrightharpoons -2$ oxidation states. The widely divergent E_m's of the two Fe-S clusters of *A. vinelandii* ferredoxin have been shown by a potentiometric titration to differ by almost 750 mV (Fig. 5). In this titration (monitored optically), the high-potential center was preoxidized with ferricyanide and then titrated reductively (318).

The observations cited above (265, 313) now explain earlier data which showed that *A. vinelandii* ferredoxin I transferred only one electron ($n = 1$) at −420 mV (313) and which drew criticism (199) based on the then prevalent er-

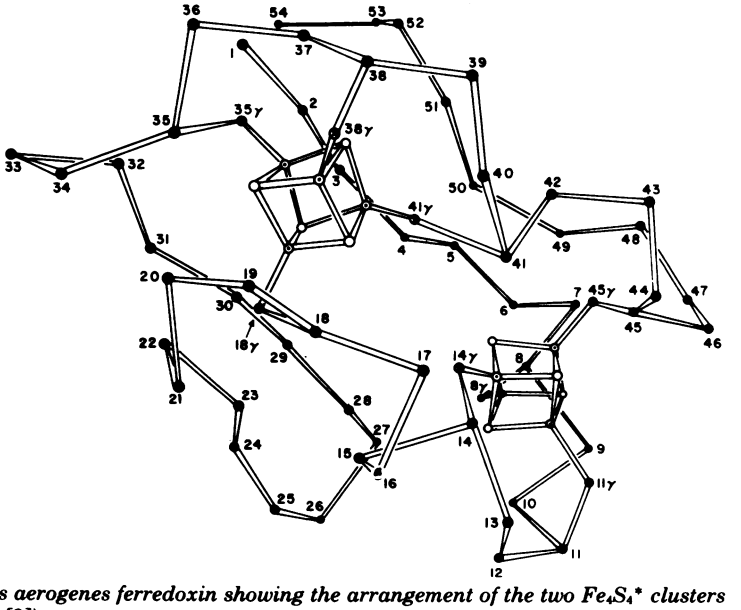


FIG. 3. *Structure of Peptococcus aerogenes ferredoxin showing the arrangement of the two Fe_4S_4* clusters on the peptide (from Adman et al. [3]).*

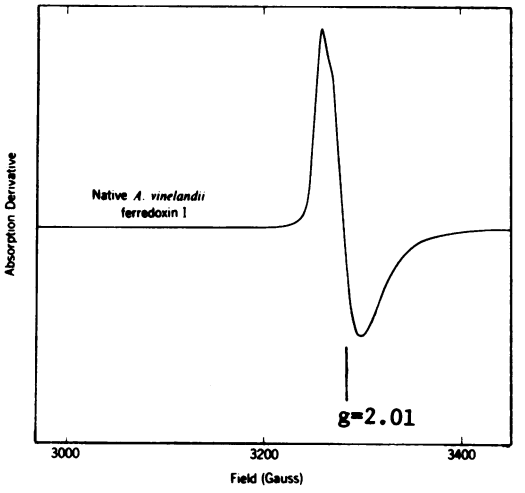


FIG. 4. *EPR spectrum of Azotobacter vinelandii ferredoxin I as it is isolated. The EPR signal is from the low-potential Fe_4S_4* center which is paramagnetic in the oxidized state; the high-potential Fe_4S_4* center is reduced (diamagnetic) here and therefore is not contributing to the EPR spectrum.*

roneous beliefs that all ferredoxins with two iron-sulfur clusters should transfer two low-potential electrons and that titration data from these ferredoxins should fit a Nernst equation in which $n = 2$.

The unusual characteristics of the *Azotobacter*-type ferredoxins, compared with clostridial-type ferredoxins, may be due to a difference in the basic protein structure. Such a difference was first suggested by experiments showing that *Azotobacter* ferredoxin I and *C. pasteurianum* ferredoxin were denatured at different rates by guanidine and sulfhydryl reagents (313). Although all cysteine residues in the amino-terminal sequence of the *Azotobacter* ferredoxin correlate with homologous residues in other ferredoxins, the appearance of a cysteine residue in position 24 is believed by Howard et al. (125) to cause a major change in the environment of one of the two iron-sulfur clusters. The unusual redox characteristics of *A. vinelandii* ferredoxin I may result from this variation in ligand arrangement.

The role of *Azotobacter*-type ferredoxins in cellular metabolism is not well understood at this time. Although the low-potential Fe_4S_4* center of this ferredoxin type functions in typical ferredoxin-requiring reactions, such as nitrogen fixation, the role of the high-potential center remains unknown. A functional role for this type of ferredoxin became even more obscure when a similar ferredoxin was isolated from chromatophore membranes of the photosynthetic bacterium *Rhodospirillum rubrum* (319). Thus, whatever the role(s) of this unusual ferredoxin in bacterial metabolism, it is not, as first believed, restricted to reactions peculiar to the aerobic bacteria.

Finally, the Fe_4S_4* ferredoxins of *D. gigas* present another unusual situation. In these ferredoxins (three have been isolated), the basic unit is a single polypeptide chain of approximately 6,000 daltons, which supports one Fe_4S_4* cluster (40). This basic unit associates to form three different ferredoxins; ferredoxins I and I' are trimers of the 6,000-dalton subunit and ferredoxin II is a tetramer. The interaction of the polypeptides in the trimer and tetramer produces proteins in which the Fe_4S_4* clusters exhibit different oxidation transitions and different E_m's (50) (Table 2).

The trimer, ferredoxin I from *D. gigas*, gives a strong $g = 1.94$ EPR signal on reduction ($E_m = -455$ mV) and a much weaker signal at $g = 2.01$ in the oxidized state, a pattern similar to that of the *Bacillus* ferredoxins. The other trimer, ferredoxin I', however, shows EPR signals of about equal intensity at both $g = 2.01$ ($E_m = -30$ mV) and $g = 1.94$ ($E_m = -430$ mV), which indicates that about an equal number of iron-sulfur centers are functioning between both $-1 \leftrightharpoons -2$ and $-2 \leftrightharpoons -3$ transitions. Finally, the tetramer, ferredoxin II, was shown on reduction to exhibit an EPR signal of high intensity at $g = 2.01$ ($E_m = -130$ mV), with a much weaker signal at $g = 1.94$. The unusual characteristics of

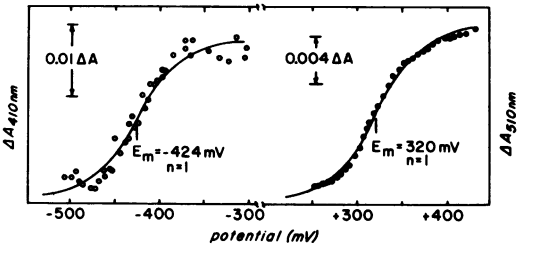


FIG. 5. *Potentiometric titration of the high- and low-potential Fe_4S_4* clusters of Azotobacter vinelandii ferredoxin I. Symbols: ●, change in absorbance at 510 nm ($\Delta A_{510\,nm}$)—reductive titration with ascorbate of the high-potential Fe-S center which had been oxidized by pretreatment with 60 μM ferricyanide; ○, change in absorbance at 410 nm ($\Delta A_{410\,nm}$)—reductive titration with dithionite of the low-potential Fe-S center. Both titrations were carried out anaerobically, and the reaction mixtures contained redox mediator dyes appropriate for the potential range over which the titration was conducted. A Pt-Ag/AgCl electrode in the titration cuvette permitted the simultaneous measurement of the absorbance and the oxidation-reduction potential (from Yoch and Carithers [318]).*

the *D. gigas* ferredoxins have been explained by "the possibility that changes in quaternary structure of the different oligomers can affect the relative stabilities of the (iron-sulfur) oxidation states of the same monomeric unit" (50). In comparing the E_m of *Chromatium* HiPIP (+350 mV) with that of *D. gigas* ferredoxin II (−30 mV) (both of which function between the −1 ⇌ −2 redox states), it is again obvious that the polypeptide both stabilizes the oxidation states and has a direct influence on the redox potential of the Fe_4S_4* cluster.

IRON-SULFUR ENZYMES

The iron-sulfur cluster (in an appropriate ligand environment) is one of the most reducing redox centers found in nature, and for this reason it is very often found as the prosthetic group in oxidoreductases where the oxidized/reduced couple of the substrate is below 0 mV. Thus, the metabolism of numerous substrates, from acetaldehyde and α-keto acids, which have reduction potentials around −550 mV, through succinate with a reduction potential near 0 mV, is catalyzed by enzymes containing iron-sulfur centers. However, just as there are a small number of high-potential iron-sulfur electron carriers, so too are there iron-sulfur-containing enzymes (involved in bacterial nitrate and nitrite metabolism) that function in this positive redox range.

Because a large number of bacterial iron-sulfur enzymes have now been isolated and are under active study, review of this subject must in some way be restricted. The discussion of these enzymes is therefore limited to a brief description of their cellular functions and biochemical characteristics and the new developments in the field. The treatment given to each enzyme generally reflects the amount of information available on that particular enzyme; in some cases, so little is known about the enzyme that one can little more than catalog its existence as an iron-sulfur-containing enzyme. The iron-sulfur enzymes have been arbitrarily grouped into several classes depending upon the nature of their electron-carrying prosthetic groups. As Table 3 shows, the least complex iron-sulfur enzymes contain no additional cofactors, whereas the most complex enzymes of this group contain the iron-sulfur complex plus three additional cofactors.

Iron-Sulfur Enzymes

Hydrogenase. Hydrogenase, perhaps the most extensively studied of all the simple iron-sulfur enzymes, is found among all the major physiological groups of bacteria, including the aerobic hydrogen bacteria, anaerobes, photosynthetic bacteria, cyanobacteria, and rhizobium root nodule bacteroids (180). The function of hydrogenase in anaerobes is to provide a type of anaerobic respiration in which excess reducing equivalents are transferred to protons with the subsequent evolution of hydrogen. These hydrogenases are bidirectional in that they catalyze both the production and the oxidation of molecular hydrogen. In contrast, H_2 uptake hydrogenases (which are unidirectional, H_2-oxidizing enzymes only) are found in the aerobic hydrogen bacteria (234), where they provide the cell with

TABLE 3. *Bacterial iron-sulfur enzymes*

Prosthetic group	Enzymes
Iron-sulfur	Hydrogenase, ω-hydroxylase, 4-methoxybenzoate-*O*-demethylase, iron-sulfur proteins bound to procaryotic photosynthetic membranes, glutamine phosphoribosyl pyrophosphate amido transferase
Iron-sulfur-thiamine pyrophosphate	Pyruvate-ferredoxin oxidoreductase
Iron-sulfur-flavin	Succinate dehydrogenase, NADH dehydrogenase, *Pseudomonas* formate dehydrogenase, dihydroorotate dehydrogenase, glutamate synthase, adenylyl sulfate reductase, trimethylamine dehydrogenase
Iron-sulfur-heme	Sulfite reductase (dissimilatory)
Iron-sulfur-molybdenum	Nitrogenase, nitrate reductase (dissimilatory), CO_2 reductase (formate dehydrogenase), iron-sulfur-molybdenum protein of unknown function
Iron-sulfur enzymes with two or more additional cofactors	Sulfite reductase (assimilatory), coliform formate dehydrogenase, xanthine dehydrogenase

reducing equivalents and initiate energy-yielding processes which permit autotrophic growth on CO_2. In the nitrogen-fixing cyanobacteria (270), photosynthetic bacteria (32), *Azotobacter* (252), clostridia (57), and *Rhizobium* bacteroids (156) the uptake hydrogenase is believed to recycle the H_2 by-product of nitrogenase as a means of conserving energy (74, 75).

Depending upon the electron carrier with which hydrogenase reacts, the enzymes may be classified as follows:

(i) Hydrogen dehydrogenase (EC 1.12.1.2)

$$NADH + H^+ \leftrightharpoons NAD^+ + \tfrac{1}{2}H_2$$

(ii) Cytochrome c_3 hydrogenase (EC 1.12.2.1)

$$\text{cytochrome } c_3 \text{ (Fe II)} + H^+ \leftrightharpoons \text{cytochrome } c_3 \text{ (Fe III)} + \tfrac{1}{2}H_2$$

(iii) Ferredoxin hydrogenase (EC 1.12.7.1)

$$\text{ferredoxin}_{red} + H^+ \leftrightharpoons \text{ferredoxin}_{ox} + \tfrac{1}{2}H_2$$

In addition, Adams et al. (2) have shown that clostridial hydrogenase can be reduced by a synthetic tetranuclear analog of ferredoxin.

Although the bidirectional hydrogenase from *C. pasteurianum* has been studied in considerable detail, there are a number of contradictory reports concerning this enzyme, not all of which have been resolved. Most workers agree that the clostridial hydrogenase is linked to ferredoxin (58, 111) and that the molecular weight of the native enzyme is 60,000 (58, 88, 191, 192); the number of subunits, however, remained in doubt until Chen and Mortenson (58) showed that the enzyme was a single polypeptide. The number of atoms of iron and sulfide per mole of enzyme has remained an open question. Nakos and Mortenson (191) and Erbes et al. (88) reported that the enzyme contained four nonheme irons and four acid-labile sulfurs; consistent with these values were the observations that the thiophenol extrusion product of hydrogenase was derived from Fe_4S_4* clusters (88, 105). Although there is general agreement that *C. pasteurianum* hydrogenase contains Fe_4S_4* clusters, there is wide disagreement on the number of these centers per molecule. This disagreement arises from the contrasting reports of Erbes et al. (88), whose hydrogenase preparation contained 4 Fe and 4 S per molecule, and Chen and Mortenson, who reported 12 Fe and 12 S per molecule. Quantitative extrusion of the Fe_4S_4* cores from this 12Fe-12S* preparation indicated that each molecule had approximately three of these tetrameric centers, consistent with the higher Fe-S content of their preparation (105). It is believed by these workers that the iron and sulfur of clostridial hydrogenase exists as three Fe_4S_4* clusters (59, 105).

Clostridial hydrogenase exhibits EPR signals in both the reduced (g = 2.079, 2.007, 1.961, 1.936, 1.908, and 1.892) and oxidized (g = 2.099, 2.046, and 2.005) forms (59, 88, 192), which are unlike the signals from other known Fe_4S_4* centers. This complexity may be due to spin-coupled centers in the hydrogenase. Because their EPR data indicated only 1.6 to 1.8 electron spins per mol of protein, Chen et al. (59) postulated that the reduced hydrogenase contains two paramagnetic $(Fe_4S_4^*)^{3-}$ centers and one diamagnetic $(Fe_4S_4^*)^{2-}$ center.

The hydrogenase of another obligate anaerobe, *Desulfovibrio vulgaris*, has also been studied in detail. The Miyazaki strain of this organism has both a soluble and a membrane-bound cytochrome c_3-linked hydrogenase (306, 307). The soluble enzyme has a molecular weight of 60,000 (306), and the particulate enzyme has a molecular weight of 89,000 (191). Although the number of irons in the soluble enzyme is unknown, the particulate species contains seven to nine iron atoms per molecule (307). Like the clostridial hydrogenase, the Hildenborough strain NCIB 8303 of *D. vulgaris* was recently reported to contain a 50,000-dalton hydrogenase consisting of a single polypeptide chain with 12 atoms each of nonheme iron and acid-labile sulfur per molecule (295). Because this hydrogenase could be removed from the cell without disrupting the cell membrane, it is believed to be located outside the cytoplasmic membrane, i.e., periplasmic (19).

A similar periplasmic, cytochrome c_3-linked hydrogenase has also been isolated from *D. gigas* (116). Its molecular weight is 89,500, and it is composed of two different subunits of molecular weights 62,000 and 26,000. It contains 12 atoms each of iron and sulfide, and, similar to the clostridial hydrogenase, quantitative extrusion of its Fe-S centers indicated three Fe_4S_4* clusters (116).

The hydrogenases of the photosynthetic bacteria *R. rubrum* (1, 107) and *Thiocapsa roseopersicina* (108) have both been reported to be bidirectional enzymes containing four Fe and four S* and to have molecular weights of about 65,000. Although the activity of the *R. rubrum* enzyme is rather low, it has been shown to couple to ferredoxin in both H_2 uptake and H_2 evolution reactions (1, 107). The bidirectional nature of this enzyme is surprising in light of the fact that the *R. rubrum* enzyme was always assumed to function only as an uptake hydrogenase (32).

The chromatophore-bound hydrogenase from

C. vinosum can apparently reduce ferredoxin (98), but loses this ability after it is solubilized by detergent (106, 135). Gitlitz and Krasna (106), who first solubilized the enzyme, reported a molecular weight of about 100,000, with two 50,000-dalton subunits. Kakuno et al. (135), however, found a molecular weight of only 70,000 and a variable subunit composition which depended on the sodium dodecyl sulfate concentration. The *Chromatium* hydrogenase contained four Fe and four S* (106, 135), and one group (135) suggested that a flavin peptide may be associated with it. The loss of ferredoxin-reducing activity and the unusual EPR characteristics (106) of *Chromatium* hydrogenase suggest the possibility that the purified form may no longer be in its native state.

An unusual characteristic of the hydrogenases from photosynthetic bacteria is that they appear to be relatively insensitive to O_2, compared with the bidirectional hydrogenases of other obligate anaerobes. Furthermore, although these hydrogenases are bidirectional in vitro, they appear to function primarily as uptake hydrogenases in vivo (311).

The unidirectional uptake hydrogenases, as a group, are not as well understood as the bidirectional hydrogenases. In the hydrogen bacteria the unidirectional hydrogenases play a critical role in the autotrophic life of the organisms. Among this group, various *Hydrogenomonas* species have long been known to couple H_2 oxidation directly to the reduction of nicotinamide adenine dinucleotide (NAD) (204, 303) for use in CO_2 fixation. The electron-carrying prosthetic group of the hydrogenases from hydrogen bacteria has remained unknown until recently, but recent evidence from at least one species, *Alcaligenes eutrophus*, indicates that it is not an iron-sulfur center, but riboflavine 5'-phosphate (FMN); 2 mol of FMN was recovered per mol of hydrogenase (235). A newly discovered uptake hydrogenase from the anaerobe *C. pasteurianum* is located in the periplasmic space and, like the bidirectional enzyme, it is reported to couple to ferredoxin; its prosthetic group remains unknown (57).

Models of catalytic action by hydrogenase which have been proposed are based upon the characteristics of the clostridial hydrogenase. In one view (88), the enzyme is thought to have a single Fe_4S_4* cluster which cycles between a fully oxidized state (with a rhombic EPR signal), a partially reduced state (EPR silent), and a fully reduced state (with a complex EPR signal) containing a single electron spin. In a different model (59), there are several Fe_4S_4* centers and another unknown center (called X) which is believed to have a more negative E_m than the fully reduced iron-sulfur centers, and it is this center X which is thought to be responsible for reducing the protons to hydrogen.

ω-Hydroxylase. The formal name of this iron-sulfur enzyme is alkane (or fatty acid), reduced NAD(P):oxygen oxidoreductase (hydroxylating), and it occurs in a number of aerobic organisms capable of metabolizing alkanes (176). The enzymatic hydroxylation of alkanes by *Pseudomonas oleovorans* has been studied extensively and was shown to require both a reductant and O_2 as substrates. Rubredoxin functions as an electron carrier coupling electrons from reduced NAD (NADH) via a flavoprotein NADH-rubredoxin reductase to the hydroxylase (214) (Fig. 6). The enzyme from *P. oleovarans* is a soluble, 2×10^6-dalton protein composed of 42,000-dalton subunits (175). Except for traces of flavine adenine dinucleotide (FAD), the active redox components appear to contain approximately four nonheme iron atoms per 2×10^6 daltons; these iron atoms exhibit EPR signals at $g = 4.3$ (rhombic iron signal) and $g = 1.94$ (the iron-sulfur signal) and a relatively strong signal at $g = 2.003$ (175).

4-Methoxybenzoate-*O*-demethylase. The biodegradation of aromatic *O*-alkyl (phenolic) esters as a source of carbon and energy is generally initiated by monooxygenase systems requiring NAD(P)H and O_2. In *P. putida* induction of this demethylase by 4-methoxybenzoic acid allows the organism to grow on this and other *O*-alkyl compounds (26). The enzyme is not very specific with regard to the substrate, in that it dealkylates a number of diverse molecules. Furthermore, in the presence of NADH and O_2 it attacks and opens the aromatic ring (24).

In *P. putida* a 4-methoxybenzoate-*O*-demethylase has been isolated as a soluble enzyme complex which consists of two different proteins; the first is an iron-sulfur flavoprotein, and the second (the terminal oxidase) is a simple iron-sulfur enzyme. The 42,000-dalton iron-sulfur flavoprotein contains FMN and an iron-sulfur center, is reduced by NADH, and functions as the reductase for the iron-sulfur enzyme (26, 28, 40). The iron-sulfur-containing demethylase accepts electrons from the reductase and functions as

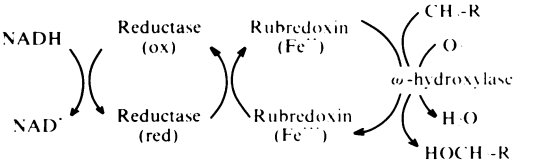


FIG. 6. *ω-Hydroxylase system of Pseudomonas oleovorans (after Peterson et al. [214]).*

the terminal oxidase. It is a 120,000-dalton protein dimer containing an Fe_2S_2* center which on reduction with NADH and the reductase exhibits EPR signals at g = 2.01, 1.91, and 1.78 (28). In its EPR characteristics it closely resembles the Rieske protein (225) except that the E_m of this center in the oxidase is reported to be about +5 mV (27), which makes it about 270 mV more reducing than all other proteins containing Reiske-type Fe-S centers. This iron-sulfur-containing demethylase not only represents a new subgroup of the oxygenases, but it is the first protein in which substrate-induced reduction of a Rieske-type iron-sulfur center has been observed.

Iron-sulfur proteins bound to procaryotic photosynthetic membranes. Iron-sulfur proteins, because of their lack of unique optical features in membranes, have only recently been discovered as important constituents of the highly pigmented (chlorophyll-containing) photosynthetic membrane. It has only been since the application of low-temperature EPR spectroscopy to photosynthetic systems that it has been possible to detect iron-sulfur centers in chlorophyll-containing membranes.

EPR analysis of membrane-bound iron-sulfur proteins was first applied to chromatophores from the purple sulfur bacterium *Chromatium*. These membranes showed two iron-sulfur centers, which, in the reduced state, had g values near 1.94 (78, 94) and could be distinguished from one another on the basis of their oxidation-reduction potentials (one iron-sulfur center had an E_m of −50 mV and the other had an E_m of −290 mV) (94). These centers have not yet been demonstrated to undergo light-dependent oxidation or reduction reactions, nor is there any information available as to a possible enzymatic activity involving these iron-sulfur centers.

In contrast to the two g = 1.94 centers in *Chromatium*, the green sulfur bacterium *Chlorobium* and all of the purple nonsulfur bacteria have three such iron-sulfur centers which are also distinguished from one another on the basis of their midpoint oxidation-reduction potentials. An example of a typical titration used to resolve these iron-sulfur centers is shown in Fig. 7, where the amplitude of the EPR signal in *R. rubrum* chromatophores was monitored at defined oxidation-reduction potentials. Table 4 summarizes the midpoint oxidation-reduction potential of the g = 1.94 centers in representative species from the three families of photosynthetic bacteria. In *R. rubrum* these centers may be related to succinate dehydrogenase, since titration of the solubilized enzyme showed redox centers with nearly identical E_m's (53). There are, however, other enzymes, such as NADH dehydro-

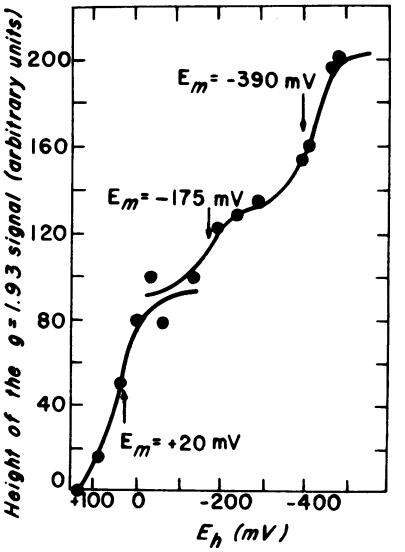


FIG. 7. *Oxidation-reduction titration of the g = 1.93 signal in Rhodospirillum rubrum chromatophores. Oxidation-reduction titrations of the membrane-bound iron-sulfur centers were performed by reducing the preparations (containing redox mediator dyes) with sodium ascorbate or sodium dithionite and monitoring the extent of reduction (as measured by the increased amplitude of the g = 1.93 signal) by EPR spectroscopy. Samples were withdrawn from the anaerobic titration vessel at fixed oxidation-reduction potentials (E_h) (as measured by a Pt-Ag/AgCl electrode) and transferred anaerobically to EPR tubes for X-band EPR analysis at 35°K (from Yoch et al. [319]).*

TABLE 4. *Oxidation-reduction properties of the g = 1.94-type iron-sulfur centers in chromatophore membranes of photosynthetic bacteria*

Family	Species	E_m (mV)	Reference
Rhodospirillaceae	*Rhodospirillum rubrum*[a]	+20, −175, −390	319
	Rhodopseudomonas capsulata	+30, −235, −335	217
	Rhodopseudomonas sphaeroides	+40, −175, −390	218
Chromatiaceae	*Chromatium vinosum*	+50, −290	94
Chlorobiaceae	*Chlorobium limicola*	−25, −175, −550	146

[a] In *R. rubrum* this EPR signal is actually at g = 1.93.

genase, which are also bound to the chromatophore and which have EPR and redox characteristics very similar to those of succinate dehydrogenase. It may be necessary to use kinetics or inhibitors to distinguish between the EPR signals induced by NADH and those induced by succinate, since both substrates reduce $g = 1.93$ iron-sulfur center(s) in *R. rubrum* chromatophores (119). That NADH dehydrogenase does reside on the chromatophore is further suggested by a distinctive EPR absorption band at $g = 2.1$ (Yoch, unpublished data), which by analogy with mitochondria (200) would be associated with centers 3 and 4 of NADH dehydrogenase.

In 1964, Rieske and co-workers (224, 225) solubilized and purified from bovine heart mitochondria via Complex III an electron-carrying protein (molecular weight, 26,000) which contained two irons and two sulfides per molecule. This protein is unusual because in its reduced state it has its major EPR absorption band at $g_y = 1.89$ ($g = 2.025$, 1.89, and 1.78), which is either an extreme value for $g_{average}$ or is a representative member of a new class of iron-sulfur proteins (the other two being the ferredoxin-type $g = 1.94$ and the HiPIP-type $g = 2.01$ centers). Although the function of the Rieske iron-sulfur center is unknown, it appears to operate near cytochrome *c* in the mitochondrial electron transport chain.

Several years ago Rieske-type iron-sulfur centers were also observed in chromatophore membranes of both the purple sulfur bacterium *Chromatium* (78, 94) and the purple nonsulfur bacteria *Rhodopseudomonas sphaeroides* (218) and *Rhodopseudomonas capsulata* (205), where they were characterized by their EPR signal at $g = 1.89$. The EPR spectrum of the reduced Rieske center in chromatophores of *R. rubrum* (Fig. 8) is representative of those centers found in other bacterial photosynthetic membranes. The E_m of the Rieske center in the purple photosynthetic bacteria ranges from +275 to +310 mV. In the green sulfur bacterium *Chlorobium*, the Rieske center was found to have an E_m of +160 mV, approximately 120 mV more negative than in the purple bacteria.

At alkaline pH (beyond pH 6.8 for the *Chlorobium* protein and pH 8 for the *R. sphaeroides* protein) the E_m of the Rieske center was found to be pH dependent. The E_m values were shifted −60 mV per pH unit, indicating that at the proper pH this protein can take up one proton along with the electron on reduction (146). Reexaminations of other Rieske centers, including that from mitochondria, have shown a similar effect of pH on the E_m (216). These findings

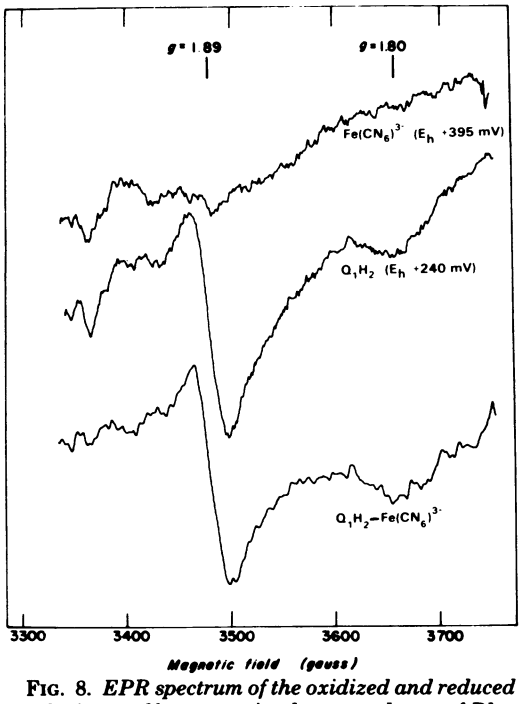


FIG. 8. *EPR spectrum of the oxidized and reduced Rieske iron-sulfur center in chromatophores of Rhodospirillum rubrum. Oxidant, $Fe(CN_6)^{3-}$, ferricyanide; reductant, Q_1H_2 hydroquinone. EPR measurements were at 20°K (Yoch, unpublished data). E_h, Oxidation-reduction potential.*

indicate that this carrier may play a role in proton translocation across the membrane, a role suggested for the Rieske protein in mitochondria even before the pH dependence was shown (207).

Given that the 2Fe-2S* Rieske center may be a proton carrier, its function in the photosynthetic apparatus is still not understood at this time. Although illumination of *Chromatium* (94) and *R. sphaeroides* (218) chromatophores at room temperature caused the prereduced Rieske center to be oxidized, these experiments provided no real indication of its physiological role in these energy-conserving systems.

Iron-sulfur proteins are also found in the cyanobacteria, where they are tightly bound to the photosynthetic membranes and are involved in a plant-type photosynthesis (95). Iron-sulfur EPR signals were first detected in lamellar particles of *Anabaena cylindrica* after illumination at 77°K, a process which oxidized the photosynthetic reaction center chlorophyll and reduced the iron-sulfur center in a manner similar to that previously observed in isolated plant chloroplasts (170). This treatment resulted in the appearance of a paramagnetic component with an

EPR spectrum similar to (but not identical to) that of the soluble Fe_2S_2* ferredoxin of this organism and showed g values at $g_x = 2.05$, $g_y = 1.95$, and $g_z = 1.87$ (95). On illumination at room temperature in the presence of dithionite, additional EPR signals at $g = 2.05$, 1.93, and 1.90 were also generated. In spinach chloroplasts these two sets of signals were designated centers A and B, respectively, and were shown to be associated with Photosystem I (170), the photosystem which generates the low-potential reducing equivalents. Both iron-sulfur centers were present in a highly specialized form of blue green algae cells called heterocysts (51), which are known to contain only Photosystem I.

The fact that the light-reduced iron-sulfur center (center A) in chloroplasts is equivalent to the amount of chlorophyll P_{700} photooxidized (based on electron spin counting) suggests that center A is the first or primary electron acceptor of Photosystem I. However, at 20°K the photoreduced iron-sulfur centers A and B in both chloroplasts and *Anabaena* fragments (51) remain in the reduced state when the light is turned off, whereas P_{700} photooxidation is reversible, suggesting that P_{700} is not linked directly to the reduction of centers A and B. There is a photo-induced signal, however, with g values at 2.08, 1.88, and 1.76 in both chloroplasts and preparations from the cyanobacterium *Chlorogloea fritschii* (89), which is reversible in the dark. This center has been called X and has been proposed to be the primary electron acceptor of Photosystem I (31, 89). This signal is believed to be due to either an iron-sulfur center (91) or an iron-quinone (31) center. At this point, the primary electron acceptor in plant-type photosynthesis (which is found in the cyanobacteria) remains a matter of controversy.

Glutamine phosphoribosyl pyrophosphate amido transferase. Glutamine phosphoribosyl pyrophosphate amido transferase catalyzes the first amination step in the synthesis of purines:

Magnesium 5-phosphoribosyl-1-pyrophosphate
+ glutamine $\leftrightharpoons$ 5-phosphoribosyl-1-amine
+ glutamate + magnesium pyrophosphate

Wong et al. (304) have shown recently that this enzyme from *Bacillus subtilis* is a tetramer of 50,000-dalton subunits and that each subunit contains approximately three irons and two sulfides. They report that the enzyme is inactivated by iron extraction or air oxidation, but is not bleached by dithionite (an observation which is at variance with all other iron-sulfur proteins). It was suggested that the iron was organized as a reduced single iron center and a fully reduced Fe_2S_2* cluster, both of which were thought to serve only as structural components (304). Additional work is obviously needed to confirm such a nonconventional role for an Fe-S center.

Iron-Sulfur-Thiamine Pyrophosphate Enzymes

Pyruvate dehydrogenase. The iron-sulfur enzymes that contain thiamine pyrophosphate (TPP) are involved in the reduction of ferredoxin by pyruvate and similar α-keto organic acids. The most thoroughly characterized example of these enzymes is the clostridial pyruvate-ferredoxin oxidoreductase (EC 1.2.7.1). In the saccharolytic clostridia, such as *C. pasteurianum* and *Clostridium butyricum*, pyruvate dehydrogenase serves as one of the key enzymes (along with glyceraldehyde phosphate dehydrogenase and NADH/NADH-ferredoxin reductase [134]) in providing glucose-fermenting cells with reduced ferredoxin for N_2, proton, CO_2, and nicotinamide adenine dinucleotide phosphate (NADP) reduction and acetyl coenzyme A (CoA) for substrate-level adenosine 5'-triphosphate (ATP) synthesis. The ferredoxin-dependent reductive carboxylation reactions catalyzed by these dehydrogenases functioning in reverse are believed to play a central role in the photoautotrophic life of the photosynthetic sulfur bacteria (43) and in providing precursors for the de novo synthesis of several amino acids by rumen bacteria (231).

The pyruvate-ferredoxin oxidoreductase from *C. acidi-urici* occurs as three isozymes, which have molecular weights of about 240,000 and contain six nonheme irons and three acid-labile sulfurs (289) (the sulfide in the enzyme is, no doubt, equimolar with the iron). The iron-sulfur center(s) of the enzyme is not reduced by pyruvate without the presence of reduced coenzyme A (CoASH), which suggests an enzyme-bound hydroxyethyl-TPP intermediate (288) and the following sequence of reactions:

pyruvate + TPP-E_{ox}
$\leftrightharpoons$ (hydroxyethyl-TPP)-E_{ox} + CO_2

(hydroxyethyl-TPP)-E_{ox} + CoASH
$\leftrightharpoons$ acetyl-CoA + TPP-E_{red}

TPP-E_{red} + Fd_{ox} $\leftrightharpoons$ TPP-E_{ox} + Fd_{red}

Summary: pyruvate + CoASH + Fd_{ox}
$\leftrightharpoons$ acetyl-CoA + CO_2 + Fd_{red}

Unlike the other cofactors found in iron-sulfur enzymes, TPP in pyruvate dehydrogenase is involved in the formation of the oxidizable sub-

strate, hydroxyethyl-TPP, but TPP itself appears to have no direct role in the electron transport reactions.

It has long been assumed that the same enzyme was responsible for both the forward reaction (the phosphoroclastic splitting of pyruvate) and the reverse reaction (pyruvate synthase) which brings about the fixation of CO_2 by a ferredoxin-linked reaction (25). A recent report, however, indicates that the two reactions are catalyzed by different enzymes (230). The pyruvate- and α-ketoglutarate-ferredoxin oxidoreductases of other bacteria (5, 22, 47, 93, 141, 213, 241) have not been well characterized.

Iron-Sulfur-Flavin Enzymes

Succinate dehydrogenase. Compared with the mitochondrial enzyme, relatively little work has been done on bacterial succinate dehydrogenase (EC 1.31.99.1), but this enzyme has been solubilized from membranes of a number of bacteria, including *Corynebacterium diphtheriae* (208), *Micrococcus lactilyticus* (301), *E. coli* (143), *R. sphaeroides* (129), *Vibrio succinogenes* (150), and *R. rubrum* (53, 68, 117). The membrane-bound succinate dehydrogenase from anaerobic bacteria serves as a fumarate reductase and acts as the terminal electron acceptor in an anaerobic electron transport system which generates ATP (149) and produces succinate. Although the membrane-bound nature of this enzyme in photosynthetic bacteria suggests that it functions as a fumarate reductase, such a role seems superfluous in an organism capable of photophosphorylation. Nevertheless *R. rubrum* chromatophores do show a potent fumarate reductase activity which is tightly coupled to the hydrogenase, provided a suitable electron carrier (a redox dye) is available (32). Assuming that the electron-carrying dye does not provide an artificial link between these two enzymes, neither the native electron carrier coupling these enzymes nor the physiological significance of this reaction is known.

Some of the earlier work had suggested that cytochrome *b* was a component of bacterial succinate dehydrogenase (197, 208); however, later studies indicated that it was similar to the succinate dehydrogenase from mitochondria and contained only flavin and iron-sulfur centers (53, 68, 117, 129, 149, 301). The enzyme solubilized from *R. rubrum* chromatophore membranes is perhaps the best characterized bacterial succinate dehydrogenase. It has a molecular weight of 85,000 (53, 68, 149) and is composed of a 60,000-dalton subunit and a 25,000-dalton subunit (68, 117). The ratio of flavin to iron to sulfide in the native enzyme is 1:8:8 (68, 149). Both flavin and nonheme iron are localized on the larger subunit, whereas the smaller subunit contains only iron and sulfide (53). The oxidized enzyme exhibits an HiPIP-type EPR signal from an iron-sulfur cluster (53) which corresponds to the center S-3 in mitochondria (196). (Iron-sulfur centers of succinate dehydrogenase are designated with an S, those of NADH dehydrogenase are designated with an N, and the numbers designate a specific center.) Reduction of the enzyme with succinate reveals another iron-sulfur center with an EPR signal at $g = 2.03$, 1.93, and 1.91 (analogous to Fe-S center S-1 in mitochondria) and a flavin semiquinone signal at $g = 2.006$ (53, 68, 119). The redox potentials of the Fe-S centers of bacterial succinate dehydrogenase can be seen from a titration of *R. rubrum* enzyme (Fig. 9). Three redox centers with E_m's of +50, −160, and −380 mV are observed; the first two redox centers probably represent iron-

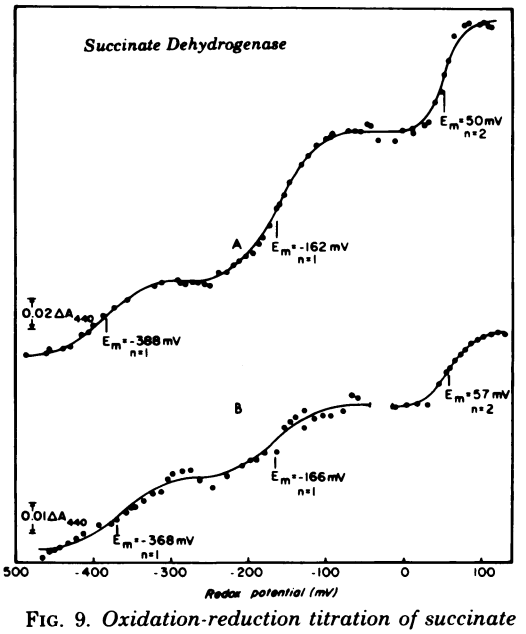


FIG. 9. *Oxidation-reduction titration of succinate dehydrogenase from Rhodospirillum rubrum. The enzyme was solubilized from chromatophore membranes with the detergent lauryl dimethylamine oxide (line A) or alkaline (pH 9.5) treatment (line B). The potentiometric titrations were performed with an anaerobic cuvette system that allowed the simultaneous measurement of absorbance changes (ΔA) and oxidation-reduction potential changes. The enzyme was titrated reductively with small additions of 30 mM sodium dithionite. Absorbance changes in the enzyme were monitored at 440 nm (each point was corrected by subtracting absorbance changes of the oxidation-reduction mediator dyes as determined by a prior titration of the mediators alone) (from Carithers et al. [53]).*

sulfur centers S-1 and S-2. The presence of a −380-mV redox center in the solubilized *R. rubrum* enzyme suggests a similarity to a mitochondrial succinate dehydrogenase center characterized by Ohnishi et al. (197), who observed a −400-mV iron-sulfur component in a solubilized preparation. In this beef heart enzyme it appears that the −250-mV S-2 center was altered on its removal from Complex III and converted to the −400-mV form; on reconstitution with the particulate cytochrome *b*·*c* complex the potential of this Fe-S center shifted back to −250 mV. The existence of both −160-mV and −380-mV centers in the detergent-solubilized *R. rubrum* enzyme (53) indicates that this preparation may have been heterogenous with respect to the S-2 iron-sulfur center. It must be noted, however, that potentiometric EPR titrations of the untreated membranes also revealed three iron-sulfur centers with these same redox potentials (Fig. 7).

The solubilized succinate dehydrogenase from *R. sphaeroides* had EPR characteristics similar to those of *R. rubrum* (i.e., a flavin free radical and S-1 and S-2 signals), but only one non-succinate-reducible iron-sulfur center (S-2) was observed in a redox titration (129).

The enzymatic mechanism of bacterial succinate dehydrogenase is presumed to be the same as that of the mitochondrial enzyme (144). It has been proposed that in this enzyme, electrons flow from succinate through center S-1 and the flavin to center S-3 and from there to the electron transport chain (196, 197). The function of center S-2 (E_m = −160 mV) remains unknown in both mitochondrial and bacterial enzymes and may be an artifact of enzyme purification (16, 196, 229), since several groups have reported variable values for electron quantitation of this center in the mammalian enzyme. On the other hand, the occurrence of eight iron atoms (enough for two 2Fe centers and one 4Fe center) and quantitative extrusion of two Fe_2S_2* centers (229) argue against center S-2 of succinate dehydrogenase being an artifact.

Reduced nicotinamide adenine dinucleotide dehydrogenase. Although NADH dehydrogenase (EC 1.6.99.3) plays an important role in energy metabolism, surprisingly little is known about the bacterial enzyme. Only a couple of bacterial NADH dehydrogenases have been isolated. The enzyme from *E. coli* (111), which was first solubilized by freeze drying membranes, is a metalloflavoprotein containing nonheme iron, acid-labile sulfur, FMN, and FAD. The mole ratio of these cofactors was not determined. Recent extensive purifications of the *E. coli* NADH dehydrogenase have shown it to be a single polypeptide chain of 38,000 daltons (66), but the flavin cofactor (FAD) was lost during the isolation process (66, 67) and again the mole ratio of iron-sulfur was not determined.

The NADH dehydrogenase from *A. vinelandii* was first recognized as an iron-sulfur protein by an EPR signal at g = 1.94, which appeared on reduction of *Azotobacter* membranes with NADH (17). The enzyme has been isolated in two forms, depending upon the amount of iron in the growth medium (69). Both forms of NADH dehydrogenase have molecular weights of 56,500, but the enzyme isolated from cells grown on low levels of iron contained as cofactors one molybdenum, two nonheme irons, and two sulfides per FMN, whereas the enzyme from cells grown on high levels of iron contained four irons and four sulfides per FMN (69). The NADH-reduced enzyme from low-iron cells exhibited a complex EPR spectrum due to Mo(V) (70) and a single iron-sulfur center with g values of 2.034 and 1.934 (69). The enzyme from normal iron growth conditions exhibited an EPR signal at g = 2.016 in the oxidized state and signals with g values at 2.042, 2.034, and 1.940 in the reduced state (69). The mechanism of this enzyme is unknown.

Although the bacterial NADH dehydrogenases appear to have the same general properties as the NADH dehydrogenase from mitochondria, our detailed knowledge of the Fe-S centers of these bacterial enzymes lags by at least an order of magnitude. At least part of the reason may be the general instability of the bacterial enzymes; also, most interest in this enzyme has been focused, for traditional reasons, on the mitochondrial enzyme.

***Pseudomonas* formate dehydrogenase.** Although most formate dehydrogenases are membrane bound, donate electrons directly to insoluble electron transport carriers, and have molybdenum and selenium as additional cofactors, the enzyme from *Pseudomonas oxalaticus* is an iron-sulfur flavoprotein (123) that catalyzes the following reaction:

$$HCOO^- + NAD + H_2O \leftrightharpoons HCO_3^- + NADH + H^+$$

With this enzyme the organism is capable of growing on formate as the sole carbon and energy source (29).

The soluble formate dehydrogenase from *P. oxalaticus* is isolated in two equally active forms (I and II) having molecular weights of 320,000 and 175,000, respectively (122). Sodium dodecyl sulfate electrophoresis has shown form I to have two subunits with molecular weights of 100,000 and 59,000; it contains 2 FMNs and 17 to 20 irons per molecule and is assumed to have the

same amount of sulfide. Formate dehydrogenase II contains 1 FMN and 8 to 10 irons. The fact that form II was not observed in the early purification steps, but appeared later, suggested the possibility that form I is a dimer of form II. The enzymatic mechanism of this iron-sulfur flavoprotein is not known, but the available data show that for the enzyme to react with either formate or NAD(H), it requires the presence of flavin (122).

Dihydroorotate dehydrogenase. This enzyme catalyzes both the synthesis and degradation of pyrimidines by the following reaction:

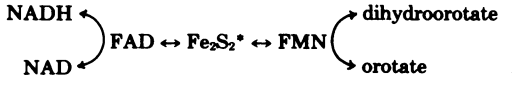
L-5,6-dihydroorotate + oxidized NAD

$$\rightleftharpoons \text{orotate} + \text{NADH} + \text{H}^+$$

Clostridium oroticum (formerly *Zymobacterium oroticum*) (56), when adapted to ferment orotic acid, synthesizes a high level of the iron-sulfur flavoprotein dihydroorotate dehydrogenase (EC 1.3.1.14), and it was from this organism that the enzyme was first purified (9). In the normal course of metabolism the enzyme presumably functions mainly in the synthesis of pyrimidines, and this reaction involves an oxidation of dihydroorotate by oxidized pyridine nucleotides rather than a reduction of orotate.

Although the enzyme has been isolated from other organisms, the most thoroughly studied example of the enzyme is that from *C. oroticum*. It has a molecular weight of 115,000 and has the unique property of containing equimolar amounts of FAD and FMN (two each) as well as four nonheme irons and four acid-labile sulfurs (7, 101, 179). Data from subunit molecular weight determinations and peptide mapping suggest that the enzyme is a tetramer composed of four identical polypeptide chains (7). It has been proposed that the protein contains two equivalent catalytic sites and that the iron atoms are grouped in Fe_2S_2* clusters (7). All of the cofactors seem to participate in the catalysis, as shown by flavin semiquinone EPR signals at $g = 2.005$ and iron-sulfur signals at $g = 2.0$, 1.94, and 1.92 in the substrate-reduced enzyme (8, 9).

The mechanism proposed for dihydroorotate dehydrogenase is as follows (7):



such that H(S) on carbon 5 is eliminated (30).

Glutamate synthase. Glutamate synthase (EC 2.6.1.53) catalyzes the following reaction:

glutamine + α-ketoglutarate + NAD(P)H

$$\rightleftharpoons 2 \text{ glutamate} + \text{NAD(P)}^+$$

This enzyme, when combined with glutamine synthetase, provides a more efficient pathway for fixing ammonia than does glutamate dehydrogenase. Nitrogen-fixing bacteria (193) and a number of coliforms (169) derepress the synthesis of these two enzymes when the level of ammonia in the medium falls below a certain level. Other bacteria, such as *Bacillus megaterium* (84) and *Caulobacter crescentus* (85), probably use this enzyme system exclusively as a means of incorporating ammonia into amino acids.

The glutamate synthase from *E. coli* is a soluble enzyme of 800,000 daltons composed of four 53,000-dalton and four 135,000-dalton subunits (178). It has been proposed that the enzyme is actually a tetramer and that the combination of one large and one small subunit contains one FAD, one FMN, eight irons, and eight sulfides (178).

The *Klebsiella aerogenes* (formerly *Aerobacter aerogenes*) glutamate synthase appears to be a simple dimer composed of a 175,000-dalton subunit and a 51,500-dalton subunit (280). The cofactors of this enzyme are reported to be 1 FAD, 1 FMN, 7 irons, and 13 sulfides per molecule. The large subunit appears to be the binding site for glutamine and to contain the cofactors (280). The enzyme is thought to catalyze the amination of α-ketoglutarate without the presence of free ammonia (103) by the following mechanism (178):

$$\text{H}^+ + \text{NADPH} + \text{E·flav} \rightleftharpoons \text{E·flav·H}_2 + \text{NADP}^+$$

$$\text{E·flav·H}_2 + \alpha\text{-ketoglutarate} + \text{Gln} \rightleftharpoons 2\ \text{Glu} + \text{E·flav} + \text{H}^+$$

Summary: NADPH + α-ketoglutarate + Gln

$$\rightleftharpoons 2\ \text{Glu} + \text{NADP}^+$$

Adenylyl sulfate reductase. Some species of sulfate-reducing bacteria, such as *Desulfovibrio* and *Desulfotomaculum*, are able to perform a type of anaerobic respiration in which they obtain energy by coupling the oxidation of H_2 and some organic compounds to the reduction of sulfate. The reducing equivalents are transferred to oxidized sulfur compounds as the terminal electron acceptors, with the production of sulfide. The first step in this process of dissimilatory sulfate reduction is the reduction of sulfate (in the form of 5′-phosphoadenylyl sulfate) to sulfite (177). The direct physiological electron donor to adenylyl sulfate reductase has not been conclusively established. However, *Desulfovibrio* contains ferredoxin, flavodoxin, and cytochrome c_3, all of which are possible electron donors; reduced methyl viologen is commonly used as the in vitro reductant.

Adenylyl sulfate reductase (EC 1.8.99.2) from *D. vulgaris* is an iron-sulfur flavoprotein with a molecular weight of 220,000 and is composed of one 20,000-dalton subunit and three 72,000-dalton subunits (37). The enzyme was determined to contain 12 irons, 12 sulfides, and 1 FAD, based on a molecular weight of 220,000. The addition of excess sulfite to an oxidized preparation having an HiPIP-type EPR signal caused a decline in the $g = 2.0$ signal, which was accompanied by a parallel rise in a $g = 1.94$ Fe-S center (212). The number and type of Fe-S clusters (2Fe or 4Fe) in adenylyl sulfate reductase are not known.

In the aerobic sulfur-oxidizing bacterium *Thiobacillus denitrificans* (35) and in the anaerobic purple sulfur photosynthetic bacterium *C. vinosum* (276), adenylyl sulfate reductase has been shown to function in the reverse direction, that is, in the oxidation of reduced sulfur molecules. In both of these organisms the electrons from the reduced sulfur molecules are used as a source of reducing equivalents for the autotrophic fixation of CO_2. The adenylyl sulfate reductases from *Thiobacillus thioparus* (211) and *T. denitrificans* (35) are also iron-sulfur flavoproteins, but the purity of these preparations was apparently not sufficient to allow a computation of the mole ratio of cofactors to protein.

Trimethylamine dehydrogenase. This enzyme has been isolated from a facultative methylotrophic organism where it catalyzes the following reaction:

$$(CH_3)_3N + H_2O + X_{ox} \leftrightharpoons (CH_3)_2NH + CH_2O + X_{red}$$

Trimethylamine dehydrogenase furnishes this methylotrophic organism with one-carbon units for utilization by either the serine or the ribose phosphate pathway and allows growth on trimethylamine. The natural electron acceptor for this enzyme is unknown, but a flavoprotein has been suggested for this role (257). For in vitro reactions, phenazine methosulfate (a flavin analog) is commonly used as the electron acceptor.

The enzyme has a molecular weight of 146,800 (257) and contains a single Fe_4S_4* cluster (120, 258) in addition to a yellow organic cofactor. Although the yellow cofactor was originally thought to be a phosphorylated pteridine derivative (258), the most recent evidence identifies it as a flavin which is substituted in a position other than in the 8-α-methylene location (259).

Chemical reduction of the enzyme with dithionite reveals a simple rhombic EPR signal ($g = 2.035$, 1.925, and 1.85) due to the iron-sulfur center having a single unpaired spin. Substrate reduction, in contrast, first generates the quinol form of the flavin, followed by an intramolecular electron transfer to the iron-sulfur group and an extensive interaction between the flavin semiquinone spin and the reduced iron-sulfur center spin (259). The formation of the spin-spin interacting species is thought to be the rate-limiting step in catalysis.

Iron-Sulfur-Heme Enzymes

Sulfite reductase (dissimilatory). The "sulfur respiratory" process, which starts with reduction of sulfate to sulfite (see discussion on adenylyl sulfate reductase above), continues with the reduction of sulfite to trithionite ($S_3O_6^{2-}$) by sulfite reductase, trithionite to thiosulfate ($S_2O_3^{2-}$) by trithionite reductase, and thiosulfate to sulfide (S^{2-}) by thiosulfate reductase (155).

The dissimilatory sulfite reductase (EC 1.8.99.1) of *Desulfotomaculum nigrificans* (formerly *Clostridium nigrificans*), which is also called bisulfite reductase because protonated sulfite is the true substrate, is a brown autooxidizable pigment which has been called P582 (281). It is a 145,000-dalton protein which contains eight atoms of iron, two acid-labile sulfurs, and a siroheme which is an octacarboxylate iron tetrahydroporphyrin. Because the molecule contains only two sulfide atoms, the release of these two sulfides along with two *g* atoms of iron per mole of enzyme by ferrous iron chelaters suggests that the iron-sulfur cluster in this enzyme is of the Fe_2S_2* type (281). The iron atoms which are not chelated are responsible for binding the enzyme inhibitor carbon monoxide. Evidence from the coliform assimilatory sulfite reductase (243) (see below) suggests that the siroheme iron that binds CO is also the site of sulfite binding. Akagi (4) has reported that ferredoxin is the physiological electron donor to this enzyme in *D. nigrificans*.

The respiratory (or dissimilatory) sulfite reductase of *Desulfovibrio* organisms is called desulfoviridin (156); it apparently is not an iron-sulfur protein (155), but it does contain a heme chromophore thought to be sirohydrochlorin (190). *D. vulgaris* does, however, contain an assimilatory reductase of 23,500 daltons which has many properties similar to those of the iron-sulfur-heme respiratory sulfite reductase of *D. nigrificans* (155), but iron and sulfide have not yet been reported in this enzyme.

Iron-Sulfur-Molybdenum Enzymes

This group of enzymes includes nitrogenase, clostridial formate dehydrogenase, respiratory nitrate reductase, and a protein of unknown function from *D. gigas*.

Nitrogenase. The most important and

widely studied of the iron-sulfur-molybdenum enzymes is nitrogenase (EC 1.7.99.2). Nitrogenase catalyzes the reduction of nitrogen gas to ammonia or, in the absence of N_2, the ATP-dependent evolution of hydrogen. The distribution of the enzyme is restricted to a relatively few species of procaryotes, i.e. *Azotobacter, Bacillus, Clostridium, Klebsiella, Rhizobium* bacteroids from leguminous plants, *Mycobacterium, Spirillum*, photosynthetic bacteria, cyanobacteria, and a few other miscellaneous organisms (324). One is struck by the fact that the nitrogenases from these various sources are remarkably similar.

Although N_2 is the normal substrate for nitrogenase, other compounds, such as nitrites, isocyanides, azides, nitrous oxide, and alkynes, are also reduced (114). One alkyne, acetylene, has been particularly useful in assays for nitrogenase because it can be detected by gas chromatography with great sensitivity (73).

Nitrogenase is composed of two different constituents; the smaller component is a simple iron-sulfur protein called the Fe protein, whereas the larger component is a more complex protein containing molybdenum as well as iron-sulfur groups and is called the MoFe protein. The number of these constituent proteins in nitrogenase is not known with certainty, but ratios of Fe protein to MoFe protein of 1:1 (80, 81, 238) and 2:1 (23, 183, 284, 294) have been reported.

Fe protein has a molecular weight of 56,000 to 67,000, is composed of two identical polypeptides, and contains four nonheme irons and four acid-labile sulfurs (201). The arrangement of the irons into a single Fe_4S_4* cluster is suggested by (i) the optical spectrum of the thiophenol-extruded iron-sulfur cluster (121) and (ii) the detection of only one species of iron atom by Mössbauer spectroscopy (82, 250). The reduced iron-sulfur cluster exhibits an EPR signal at g = 2.06, 1.94, and 1.87 (82, 203, 205) after the uptake of 0.2 to 0.8 electrons (203, 205, 251). Magnesium-ATP alters the characteristics of the iron-sulfur cluster of Fe protein in the following ways: (i) the E_m is shifted from −294 to −402 mV (325); (ii) the symmetry of the EPR spectrum becomes more axial (81, 193, 203, 251, 323, 326); and (iii) the irons become more sensitive to an iron chelater (298, 299) and a thiol reagent (278, 285). There are apparently binding sites for two magnesium-ATP's on the Fe protein (277, 286, 323, 326).

MoFe protein has a molecular weight of 200,000 to 230,000 and is composed of four subunits (201), which are large enough to be seen in electron micrographs of the protein (256). In *A. vinelandii* and most other organisms, the MoFe protein subunits are present in an $\alpha_2\beta_2$ structure (201), but the protein from *Rhizobium japonicum* is apparently a tetramer of identical subunits (130). The cofactor content of MoFe protein is not precisely known because preparations are frequently contaminated with denatured proteins which have a lowered metal content and also because analytical errors prevent the absolute precision needed in quantitating the large numbers of iron and sulfides in this protein. Some examples of reported values are shown in Table 5.

The organization of these cofactors in MoFe protein is not precisely known, but there is some information available on this point. First, Mössbauer spectroscopy suggests that the iron atoms are grouped into at least three and possibly four distinct units (82, 250). Only one center, accounting for eight iron atoms, changes its properties during turnover of the enzyme (189, 251). Second, Stasny et al. (256) have suggested from data based upon electron micrographs that each of the four subunits of MoFe protein has an electron-dense region of heavy metals near its center.

A more recent approach in studying the distribution of metals in MoFe protein has been to isolate the enzymatically active cofactor intact (Mössbauer and EPR spectroscopy were used to determine whether the cofactor was in its native state). This iron-molybdenum-containing cofactor was isolated as a low-molecular-weight unit called FeMoCo (220, 237). Although some of the iron atoms in MoFe protein appear to be in Fe_4S_4* clusters which are distinct from FeMoCo (220, 237), FeMoCo itself contains Fe, S, and Mo in a ratio of 8:6:1 such that either MoFe protein has two FeMoCo units and each FeMoCo contains one molybdenum or, alternatively, FeMoCo contains two molybdenums (220).

MoFe protein has also been extensively studied by using EPR techniques. The dithionite-reduced state of the protein exhibits EPR resonances near g = 4.3, 3.7, and 2.01 (201), but the

TABLE 5. *Cofactor content of nitrogenase MoFe protein*

Organism	No. of:			Reference
	Mo	Fe	S^{2-}	
Chromatium vinosum	2	14	11	96
Clostridium pasteurianum	2	24	24	126
	2	24	6	181
	1	14	16	64
	2	20	20	183
Azotobacter vinelandii	1.5	24	20	145
	2	24	24	189
Klebsiella pneumoniae	1	17	17	82
Rhizobium bacteroids	1.3	29	26	130

exact value depends upon the origin of the protein and the pH (251). FeMoCo has an EPR spectrum similar to that of MoFe protein (220). Isotope substitutions with ^{95}Mo and ^{57}Fe (96, 183, 189, 205, 251) suggest that the EPR resonance lines observed in the FeMo protein arise from iron atoms and not from the molybdenum. The irons are thought to be arranged in a complex which has at least three irons with a net spin of S = 3/2 (189, 205, 251) and contains two unpaired electrons per MoFe protein (189) or one unpaired electron per molybdenum in FeMoCo (220). The reduced state of MoFe protein characterized by the EPR signal at $g = 4.3$, 3.8, and 2.01 is not capable of reducing the substrates of nitrogenase, but instead MoFe protein must be reduced further by electrons from Fe protein to produce a "super-reduced" state of MoFe protein; this reduction is accompanied by a loss of the EPR signals (96, 183, 184, 203, 251, 323) and the uptake of four additional electrons (184, 300).

The enzymatic mechanism of nitrogenase is shown in Fig. 10. In this scheme, electrons from reduced ferredoxin enter nitrogenase on the Fe protein component, which then combines with two magnesium-ATP molecules (277, 284, 323, 326). The reaction of the reduced Fe protein with magnesium ATP causes a shift in the E_m of the Fe_4S_4* cluster of Fe protein from −294 to −402 mV (325). The magnesium-ATP-Fe protein complex transfers electrons to MoFe protein with the splitting of the γ-phosphate from ATP to given an optimum efficiency of one electron transferred for two ATPs split (160, 302). The operational midpoint oxidation-reduction potential of the MoFe protein is not known with certainty, but it must be more negative than −260 mV because the EPR signals develop (in part) with this E_m (6), whereas the transition to super-reduction exhibits a loss in paramagnetism. It is tempting to attribute the −460-mV E_m observed for the overall nitrogen-fixing reaction (92) to the super-reduced state of MoFe protein.

Nitrate reductase (dissimilatory). Respiratory nitrate reductase (EC 1.9.6.1), like respiratory sulfite reductase, is an enzyme used by anaerobic bacteria to dispose of excess reducing

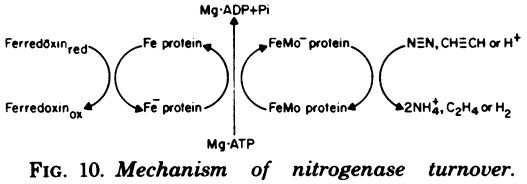


FIG. 10. *Mechanism of nitrogenase turnover. ADP, Adenosine 5'-diphosphate; Pi, inorganic phosphate.*

equivalents by depositing them on an inorganic ion. Because this enzyme is membrane bound and linked to the electron transport chain through cytochromes, the solubilized enzyme may or may not be found to contain hemes as a subunit; we have chosen to include the enzyme in this section with the iron-sulfur-molybdenum enzymes because the cytochrome may be an adventitious component.

The respiratory-linked nitrate reductase solubilized from *K. aerogenes* by deoxycholate appears in two forms, nitrate reductase I and nitrate reductase II (227). Nitrate reductase I is composed of three different subunits of 117,000, 57,000, and 52,000 daltons in a ratio of about 1:1:2. Nitrate reductase II is smaller and appears to be missing the two brown 52,000-dalton subunits which have been proposed to couple electron flow to cytochrome b_{559} in this organism (227). The cofactors in nitrate reductase I are 0.24 Mo, 8 Fe, and 8 S^{2-}, and, in addition, there are four irons not bound to sulfide (226). These cofactors are responsible for EPR signals due to Mo(V) and an iron-sulfur cluster(s) of unknown symmetry (at $g = 2.015$) in the oxidized enzyme and Mo(III) and ferredoxin-like iron-sulfur signals ($g = 2.05$, 1.95, and 1.88) in the reduced enzyme.

In *E. coli* the respiratory nitrate reductase has been solubilized by various techniques, and the resulting enzyme has different properties in each case (164). The enzyme appears to contain three different subunits; subunit *A* has a molecular weight of 142,000 and contains the catalytic site, subunit *B* has a molecular weight of 58,000 and appears to be the membrane attachment site, and subunit *C* (the cytochrome *b* peptide), when present, has a molecular weight of 19,500 (86, 164, 166–168). Evidence that the *B* subunit is involved in membrane attachment is that solubilization of the enzyme by heat treatment appears to be the result of proteolysis, and it is the *B* subunit which is degraded without loss of enzymatic activity (164, 166). The necessity for the cytochrome *b* component of the *E. coli* enzyme is questionable since many of the reported preparations do not contain hemes (99, 140, 164, 166, 168).

If it is assumed that the core enzyme consists of one *A* and *B* subunit (61, 168), the cofactor content from two different preparations has been reported to be 1 Mo, 12 Fe, and 12 S^{2-} (164) and 1.5 Mo, 20 Fe, and 19 S^{2-}, respectively (99). Both the molybdenum and iron-sulfur clusters of the enzyme have been studied by EPR techniques. Oxidized nitrate reductase exhibits an EPR signal at $g = 2.005$ due to iron-sulfur clusters and a complex signal near $g = 1.988$ due to Mo(V) (71), but the complexity of the molyb-

denum signal [presumably due to Mo(V)—,H' hyperfine splitting] was eliminated by recording the spectrum in D_2O, which revealed a simple rhombic symmetry with g values of 1.999, 1.985, and 1.964 (38). Reduction of the enzyme was accompanied by a loss of the signal from molybdenum (38) and the appearance of two different EPR signals from the iron-sulfur clusters (71). The type I iron-sulfur signal exhibited g values of 2.047, 1.889, and 1.861, whereas the type II signal had peaks at $g = 2.030$ and 1.942 (71).

The nitrate reductase of *Micrococcus denitrificans* has also been solubilized and studied. This 160,000-dalton enzyme contains 0.4 Mo, 8 Fe, and 8 S^{2-} (100, 153) and exhibits EPR signals (100) similar to those of the *E. coli* enzyme.

CO_2 reductase (formate dehydrogenase). Ferredoxin-dependent formate dehydrogenase in clostridia is believed to function primarily in the reduction of CO_2 to formate (273). Because of its physiological function, this enzyme is commonly called CO_2 reductase or more correctly, ferredoxin-CO_2 oxidoreductase. Although the enzyme in *C. pasteurianum* has always been known to be extremely sensitive to oxygen (a trait common to many Fe-S proteins), it has only recently been isolated and shown to contain iron, sulfide, and molybdenum (232). These cofactors occur in a ratio of 24:24:1 based on a molecular weight of 118,000 for the enzyme, which is composed of two subunits with molecular weights of 34,000 and 86,000. Although EPR data are not yet available to implicate the iron and sulfide in the electron transfer process, there is evidence for the participation of Mo in the catalytic mechanism, which comes from the observation that both the synthesis and activity of CO_2 reductase require the presence of molybdenum in the growth media (273).

Clostridium thermoaceticum also contains CO_2 reductase, but unlike the CO_2 reductase of *C. pasteurianum*, the enzyme from this organism uses reduced nicotinamide adenine dinucleotide phosphate (NADP) rather than ferredoxin as a reductant (12). Furthermore, the cells require both molybdenum and selenium for the formation of the enzyme. Although the CO_2 reductase from *C. thermoaceticum* has been purified (it is composed of several soluble isozymes of about 300,000 daltons), there is, as yet, no indication that it is an iron-sulfur enzyme.

Iron-sulfur molybdoprotein of unknown function. An iron-sulfur molybdoprotein has been isolated recently from *D. gigas* by Moura et al. (185); it showed no activity when assayed for either sulfite reductase activity or formate dehydrogenase activity. The protein is a single 120,000-dalton polypeptide containing 1 molybdenum, 20 nonheme irons, and 20 acid-labile sulfurs. The iron-sulfur groups appear to be Fe_2S_2* clusters which give two types of EPR spectra, depending on the temperature at which the EPR spectrum is recorded. One center, which can be resolved at relatively high temperatures (70°K), has a rhombic signal with g values of 2.02, 1.94, and 1.93. Below 55°K a second iron-sulfur signal is observed with an additional absorption band at $g = 2.06$ (185). The molybdenum component of this protein has a complex signal near $g = 1.98$. Although the biological function of this protein remains unknown, it shows certain physical similarities to xanthine oxidase.

Iron-Sulfur Enzymes with Two or More Additional Cofactors

Sulfite reductase (assimilatory). Unlike the respiratory sulfite reductase of the sulfur-reducing bacteria, the assimilatory enzyme reduces sulfite all the way to sulfide in a single, six-electron transfer step in which no intermediate compounds are released. Hydrogen sulfide is used by the cell for the biosynthesis of iron-sulfur cofactors and sulfur-containing amino acids and does not accumulate. The most thoroughly studied bacterial assimilatory sulfite reductase is the enzyme from *E. coli* described in an elegant series of experiments carried out by Siegel and his co-workers (97, 242-245). This soluble 680,000-dalton protein is composed of eight 60,000-dalton polypeptides and four 55,000-dalton subunits with four FAD and four FMN groups distributed over the eight large subunits. A total of 16 nonheme irons, 14 acid-labile sulfurs, and 4 sirohemes are bound to the four smaller subunits (97, 242, 245). The enzyme can be separated by urea into two functional "subenzymes," with partial reactions; these are a flavoprotein which contains the FAD and FMN and a hemoprotein containing the Fe-S and heme groups (242). The flavoprotein only shows nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) diaphorase and transhydrogenase activities. The FAD is thought to cycle between the fully oxidized and the fully reduced states, whereas FMN cycles between the fully reduced and the semiquinone forms (244). The hemoprotein catalyzes the reduction of SO_3^{2-} to H_2S but only with reduced methyl viologen as an electron donor (NADPH does not serve as an electron donor to hemoprotein).

The iron and sulfide of sulfite reductase reduced by NADPH show a typical $g = 1.94$ EPR signal and appear to exist in Fe_4S_4* clusters, since there is one unpaired electron spin pro-

duced for every four Fe/S (228). The hemoprotein subenzyme is believed to contain a single Fe_4S_4* center and a single heme per 55,000-dalton subunit. In summary, it has been proposed that the intramolecular electron flow in sulfite reductase is as follows:

$$NADPH \rightarrow \underbrace{FAD \rightarrow FMN \rightarrow Fe/S \rightarrow heme}_{\text{sulfite reductase}} \rightarrow SO_3^{2-}$$

However, it is also pointed out that the data are consistent with a number of branched electron flow schemes (228).

Coliform formate dehydrogenase. During anaerobic growth of *E. coli* in the presence of nitrate, a process known as nitrate respiration occurs and nitrate functions as the terminal acceptor of electrons from formate. Electron flow from formate is mediated by a membrane-bound enzyme complex that includes formate dehydrogenase, quinone, and nitrate reductase (87). The coliform formate dehydrogenase, unlike the *Pseudomonas* enzyme, does not contain flavin; instead, it contains heme, molybdenum, and selenium as cofactors (86, 87), making it perhaps the most complicated of all metalloenzymes. The formate dehydrogenase from *E. coli* is solubilized from the membrane with cytochrome *b* as a subunit (87, 159, 236). The molecular weight of the enzyme is about 590,000, and the enzyme is composed of 110,000-, 32,000-, and 20,000-dalton peptides, which are present in a ratio of approximately 1:1:1 (87). The cofactors are in the relative ratio of approximately 1 heme to 1 molybdenum to 1 selenium to 14 nonheme irons to 13 acid-labile sulfurs. Because there is one heme for approximately 154,000 daltons, the enzyme must exist as a basic tetramer; i.e., the enzyme contains four copies of each of three subunits. The selenium is covalently bound to the largest subunit, but it is not yet known if selenium participates in enzyme catalysis. The electron transport pathway from formate to nitrate can be depicted as follows:

$$HCOOH \rightarrow \underset{\text{formate dehydrogenase}}{(\text{heme, Se, Mo, Fe/S})} \rightarrow \text{quinone} \rightarrow \underset{\text{nitrate reductase}}{(\text{Mo, Fe/S, heme})} \rightarrow NO_3^-$$

The order in which the electron-carrying cofactors in the two enzymes are arranged is not intended to indicate the order in which they transfer electrons in the enzyme, as little is known on this point.

Xanthine dehydrogenase. Unlike the more thoroughly studied milk xanthine oxidase, the enzyme from anaerobic bacteria does not require oxygen and is therefore a dehydrogenase instead of an oxidase (254). The reaction catalyzed by the bacterial xanthine dehydrogenase (EC 1.2.1.37) is as follows:

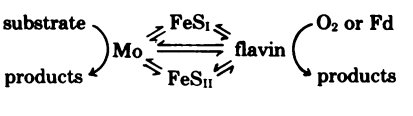
$$\text{xanthine} + X_{ox} \rightleftharpoons \text{uric acid} + X_{red}$$

Evidence has been presented which suggests that xanthine dehydrogenase in anaerobic bacteria functions as a ferredoxin reductase; thus, X in the equation above would represent ferredoxin (254, 291).

The soluble xanthine dehydrogenase from *M. lactilyticus* (formerly *Veillonella alcalescens*) has a molecular weight of about 250,000 and contains as cofactors Fe, S, FAD, and Mo in a ratio of 8:8:2:2 (65, 254). These values are similar to those found for milk xanthine oxidase and chicken liver xanthine dehydrogenase. Because the bacterial enzyme is insensitive to inhibition by cyanide (254), the presence of a persulfide group, as found in the milk enzyme (171), was questioned; however, Dalton et al. (65) reached a different conclusion based on EPR studies. Although the oxidized enzyme apparently contains Mo(VI), substrate reduction leads to the rapid appearance of Mo(IV) and Mo(V) (with EPR signals at $g = 1.99$ and 1.968), flavin free radicals ($g = 2.004$), and reduced iron-sulfur clusters with axial symmetry ($g = 2.026$, 1.94, and 1.925).

The presence of xanthine dehydrogenase in various clostridia and pseudomonads allows these bacteria to grow on purines as a source of carbon and nitrogen. The enzyme from *Clostridium cylindrosporum* (36) contains as cofactors Fe, FAD, and Mo in a ratio of 8:3:1. Xanthine dehydrogenase from *Pseudomonas acidovorans* (247) was shown to be a 275,000-dalton protein composed of two 81,000-dalton and two 63,000-dalton subunits.

The enzyme mechanism of the bacterial xanthine dehydrogenase is presumably similar to that of the milk enzyme. The milk xanthine oxidase is thought to utilize the persulfide group in a nucleophilic attack of the aromatic ring (83), with simultaneous transfer of electrons to molybdenum, which changes between the Mo(VI) and Mo(IV) oxidation states (198). The two iron-sulfur clusters of the milk enzyme serve to buffer the electron transfer between the molybdenum center and FAD where oxygen (or electron carriers in the case of bacteria) removes the electrons from the FAD anion radical (FAD·) (198). Although the intramolecular electron transfer occurs too rapidly to be measured, the steady-state electron distribution appears to follow from the E_m's of the centers, and one possible mechanism is shown below:



FERREDOXIN-DEPENDENT REACTIONS

Over the last 15 years the elucidation of the anaerobic biochemical processes carried out by the clostridia have essentially been studies of reactions catalyzed by iron-sulfur proteins. Initially, nitrogenase and all of the reactions feeding electrons to this enzyme (through ferredoxin) were shown to be iron-sulfur proteins (45, 290, 320). These enzymes include pyruvate dehydrogenase, hydrogenase, xanthine dehydrogenase, and formate dehydrogenase. The importance of iron-sulfur proteins in electron transport is seen in the reconstituted pyruvate dehydrogenase-nitrogenase system of *C. pasteurianum*, where these proteins form an anaerobic electrical conduit composed strictly of iron-sulfur centers (Fig. 11). In the obligate anaerobes, such as the clostridia and *Desulfovibrio*, the physiological reactions of the cell all operate at negative oxidation-reduction potentials. In this environment, the iron-sulfur proteins with their generally negative E_m's are especially well suited to operate as enzymes and electron carriers between the enzyme systems of catabolism and anabolism.

Clostridia

Ferredoxin plays a central role in almost every aspect of the redox-related metabolism of the clostridia. Its role in hydrogen and nitrogen metabolism was mentioned above, but it is also involved in purine, sulfur, and one-carbon metabolism. The central position of ferredoxin in linking the catabolic reactions of fermentation to the biosynthetic reactions of *C. pasteurianum* is shown in Fig. 12.

The reduction of ferredoxin coupled to the phosphoroclastic cleavage of pyruvate by the glucose-fermenting saccharolytic clostridia has long been known to be a mechanism of ferredoxin reduction (182), but only in recent years has the role of NAD-linked glyceraldehyde phosphate dehydrogenase and NADH-ferredoxin oxidoreductase been established as an equally important mechanism for the reduction of ferredoxin (134). This reaction is summarized as follows:

$$\text{glucose} \rightarrow\rightarrow \underset{\text{phosphate}}{\text{glyceraldehyde}}$$

$$\xrightarrow[\text{phosphate dehydrogenase}]{\text{glyceraldehyde}}$$

$$\text{NADH} \xrightarrow[\text{reductase}]{\text{NADH-Fd}} \text{Fd}_{\text{red}}$$

In *Clostridium kluyveri*, an organism which grows on ethanol, acetate, and CO_2, reduced ferredoxin is generated solely via NADH or NADPH, which couples electron flow to ferredoxin via an NAD(P)H-ferredoxin oxidoreductase (133). *C. acidi-urici, C. cylindrosporum, V. alcalescens*, and numerous other obligate anaerobes generate reduced ferredoxin by reactions coupled to purine degradation (297). These reactions include the oxidation of such purine degradation products as xanthine, hypoxanthine, and 8-hydroxypurine. An example of this type of purine degradation coupled to ferredoxin reduction is shown in the following equation:

$$\text{guanine} \xrightarrow[\text{guanine deaminase}]{NH_4^+} \text{xanthine} \xrightarrow[\text{dehydrogenase}]{\text{xanthine}} \text{Fd}_{\text{red}} + \text{uric acid}$$

Formate oxidation also provides numerous anaerobic bacteria with reduced ferredoxin to be used for biosynthetic processes. Formate is available to these anaerobes as a by-product of fermentation. Ferredoxin-linked formate dehydrogenase has been found in *C. pasteurianum* (290) and *C. acidi-urici* (138). Other anaerobes, such as *C. thermoaceticum* (271) and the S organism, a syntroph isolated from *Methanobacillus omelianskii* (221), reduce ferredoxin from formate via a pyridine nucleotide as follows:

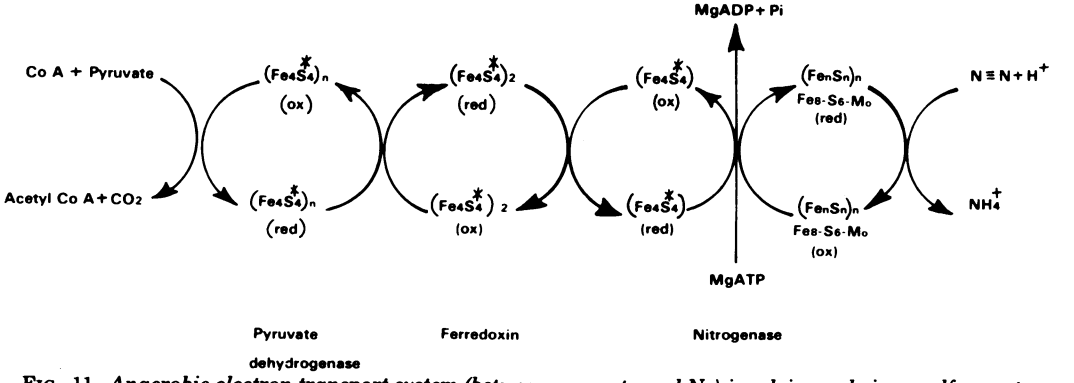


FIG. 11. *Anaerobic electron transport system (between pyruvate and N_2) involving only iron-sulfur centers. ADP, Adenosine 5'-diphosphate; Pi, inorganic phosphate.*

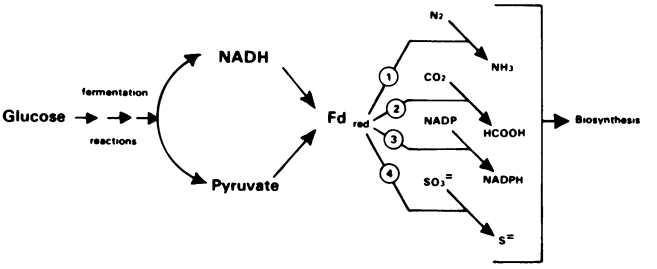


FIG. 12. *Ferredoxin-dependent metabolism of C. pasteurianum growing on glucose. Enzyme 1, nitrogenase; enzyme 2, reduced ferredoxin-CO_2 oxidoreductase; enzyme 3, reduced ferredoxin-NADP oxidoreductase; enzyme 4, sulfate reductase.*

$$\text{formate} \xrightarrow[\text{dehydrogenase}]{\text{formate}}$$

$$\text{NAD(P)H} \xrightarrow[\text{reductase}]{\text{NAD(P)H-Fd}} \text{Fd}_{\text{red}}$$

In considering the enzyme reactions of the clostridia that oxidize ferredoxin, the biosynthetic reactions, such as N_2, CO_2, SO_3^{2-}, and NO_2^- reduction, will be discussed. However, before these biosynthetic reactions are discussed, another ferredoxin-oxidizing reaction vital to the survival of these obligate anaerobes must be discussed, and that is H_2 evolution. The fermentative growth on carbohydrates generates excess reducing equivalents (reduced ferredoxin) which are disposed of by hydrogenase, which catalyzes the reduction of H^+ to H_2 as follows:

$$\text{Fd}_{\text{red}} + 2\text{H}^+ \xleftrightarrow{\text{hydrogenase}} \text{H}_2 + \text{Fd}_{\text{ox}}$$

When the ferredoxin in this metabolic reaction is reduced by pyruvate or xanthine dehydrogenase, we have yet another example of an anaerobic electron transport chain composed predominantly of iron-sulfur centers. The physiological significance of hydrogenase operating in the reverse direction to reduce ferredoxin is a matter of speculation, and in this vein one can well imagine this enzyme functioning in this direction to modulate the internal oxidation-reduction state of a cell under conditions of carbohydrate limitation.

Both NAD and NADP can be reduced by H_2 in extracts of the various clostridia (290), but these reactions appear to be of limited physiological significance, as ATP formation is obligatorily linked to H_2 formation in these organisms. NADP reduction in the clostridia is catalyzed by NADPH-ferredoxin reductase, and this enzyme preferentially catalyzes electron transfer from reduced ferredoxin to NADP, thus making it an anabolic process (134, 275).

Ferredoxin-linked CO_2 fixation in various species of clostridia has received considerable attention in recent years (44, 274). The first evidence that reduced ferredoxin could drive the reductive synthesis of pyruvate from acetyl-CoA and CO_2 was presented by Bachofen et al. (14), who used cell-free extracts of *C. pasteurianum* in a reaction presumed to be catalyzed by pyruvate-ferredoxin oxidoreductase (pyruvate dehydrogenase). The reaction showing the carboxylation of acetyl-CoA with electrons from reduced ferredoxin is as follows:

$$\text{acetyl-CoA} + \text{CO}_2 + \text{Fd}_{\text{red}} \rightarrow \text{pyruvate} + \text{CoA} + \text{Fd}_{\text{ox}}$$

Although this reaction is of dubious physiological significance to *C. pasteurianum*, it is essential for the synthesis of pyruvate by *C. kluyveri*, an organism which grows on acetate, ethanol, and bicarbonate.

Other miscellaneous biosynthetic reactions catalyzed by reduced ferredoxin include the synthesis of formate catalyzed by CO_2 reductase (this enzyme appears to be the formate dehydrogenase functioning in reverse). This reaction has been shown to occur in *C. pasteurianum* (132) and *C. acidi-urici* (272). The enzyme(s) which catalyzes the reduction of hydroxylamine and nitrite to ammonia in *C. pasteurianum* is also reported to be ferredoxin dependent (292), but it has not yet been isolated. In *Clostridium perfringens* the reduction of nitrate to nitrite is catalyzed by a ferredoxin-dependent nitrate reductase (60). NADH appears to function as the reductant precursor for the reaction sequence by utilizing NADH-ferredoxin reductase, as shown in the following reaction:

$$\text{NADH} \xrightarrow[\text{reductase}]{\text{NADH-Fd}} \text{Fd}_{\text{red}} \xrightarrow[\text{reductase}]{\text{nitrate}} \text{NO}_3^- \rightarrow \text{NO}_2^-$$

Desulfovibrio

The growth of *Desulfovibrio* on H_2 and sulfate as the sole energy source (255) suggests a role for low-potential electron transport carriers such as ferredoxins and indicates that ATP generation in these organisms can be coupled to elec-

tron transport. In discussing the ferredoxin-dependent reactions of these anaerobic sulfate-reducing bacteria, it should be recalled that they all appear to have Fe_4S_4* ferredoxins (40, 50, 322) and that the ferredoxin from *D. gigas* is a 6,000-dalton Fe_4S_4* protein which functions in several different polymeric forms, each with a different set of redox characteristics (40, 50, 187). These sulfate-reducing bacteria also contain a low-potential *c*-type cytochrome (cytochrome c_3), which in *D. gigas* complexes with the tetrameric form of ferredoxin (186); together these catalyze a number of important energy-related reactions.

The role of ferredoxin in the critical sulfur metabolism of *Desulfovibrio* was shown by LeGall and Dragoni (157), who demonstrated that ferredoxin was required for the transfer of electrons from the H_2/hydrogenase cycle to sulfate. Suh and Akagi (263) extended this observation by showing that sulfate reduction in *D. vulgaris* by H_2 required both ferredoxin and cytochrome c_3. Of the three enzymes involved in this reaction, dissimilatory sulfite reductase, trithionite reductase, and thiosulfate reductase, only the last has been shown to accept electrons from ferredoxin. These reactions are shown in Fig. 13.

Another reaction of *Desulfovibrio* requiring both cytochrome c_3 and ferredoxin is the phosphoroclastic cleavage of pyruvate coupled to the evolution of H_2 (5). This sequence is also shown in Fig. 13.

Both 2,4-dinitrophenol inhibition of sulfate reduction by H_2 (210) and growth experiments (255) strongly suggest that (ferredoxin-mediated) dissimilatory sulfate reduction is coupled to phosphorylation; however, the site of energy conservation has not yet been determined.

Moura et al. (187) have suggested that in *D. gigas* a single 6,000-dalton ferredoxin can service both pyruvate dehydrogenase ($E_m \simeq -600$ mV) and the thiosulfate reductase system ($E_m \simeq -110$ mV) because the monomeric ferredoxin can polymerize into three different forms, with the Fe_4S_4* cluster stabilizing in three very different oxidation-reduction states depending on the polymer formed. Thus, the trimer (ferredoxin I), which stabilizes almost exclusively in the $-2 \rightleftharpoons -3$ oxidation states with an $E_m \simeq -455$ mV, functions better than other forms of the ferredoxin in coupling electron flow from pyruvate dehydrogenase. The tetramer (ferredoxin II), on the other hand, which stabilizes in the $-1 \leftrightharpoons -2$ state, has an $E_m \simeq -130$ mV and is most efficient in the reduction of sulfite to sulfide (187) (Fig. 13).

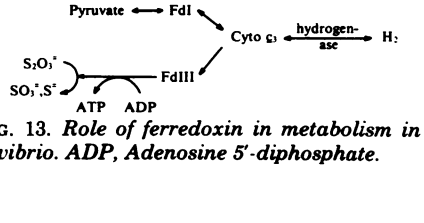


FIG. 13. *Role of ferredoxin in metabolism in Desulfovibrio. ADP, Adenosine 5'-diphosphate.*

Photosynthetic Bacteria

Ferredoxin is found in all photosynthetic bacteria as both a soluble and a membrane-bound electron carrier (311). Although ferredoxin plays a central role as a reductant in many of the reactions vital to photosynthetic bacteria, such as H_2 metabolism, N_2 fixation, pyridine nucleotide reduction, and CO_2 fixation, there is little or no information as to its mode of reduction. Only in *Chlorobium limicola* (formerly *Chlorobium thiosulfatophilum*) has it been possible to demonstrate the formation of reduced ferredoxin in a cell-free light-dependent reaction (93). The lack of evidence for the direct light-dependent reduction of ferredoxin by the purple bacteria is consistent with evidence that the primary electron acceptor in the photosynthetic process of these bacteria has an oxidation-reduction E_m of approximately −150 mV (79), far too electropositive to reduce ferredoxin, whose $E_m \simeq -400$ mV. The photoreduction of NAD ($E_m \simeq -320$ mV) by chromatophores of these organisms is believed to occur via reverse electron flow in which ATP or the energized membrane drives electrons against the thermodynamic gradient from succinate (the electron donor) to NAD. The fact that uncouplers (agents that block energy transduction but not cyclic electron transport) inhibit the photoreduction of NAD supports this theory (139). The mechanism of ferredoxin reduction therefore remains an enigma.

Although the reduction of ferredoxin by photosynthetic bacteria is not understood, it is known to couple H_2 uptake to NAD reduction (46) and to couple electron flow to the nitrogenases of *Chromatium* (312) and *R. rubrum* (314). Of a four-iron ferredoxin and an eight-iron ferredoxin found in *R. rubrum* (315), the artificially reduced eight-iron protein was several times more effective in coupling electron flow to nitrogenase (314).

Ferredoxin-linked CO_2 fixation has also been demonstrated in numerous photosynthetic bacteria and other obligate anaerobes. Reduced ferredoxin catalyzes what is believed to be a reversal of the phosphoroclastic reaction for the synthesis of pyruvate from acetyl-CoA and CO_2 (14). Other acyl-CoA derivatives, including succinyl-CoA (90), propronyl-CoA (43), and phenylacetyl-CoA (104), have been demonstrated to undergo reductive carboxylation by various enzyme preparations from photosynthetic bacte-

ria. The following equation summarizes these reactions:

$$\text{acyl-CoA} + Fd_{red} + CO_2 \rightarrow \alpha\text{-keto acid} + \text{CoA} + Fd_{ox}$$

The ferredoxin-dependent carboxylations of acetyl-CoA and succinyl-CoA, along with two other carboxylating enzymes, isocitrate dehydrogenase and phosphoenolpyruvate carboxylase, form the basis of a CO_2 fixation cycle called the reductive carboxylic acid cycle (44). In at least one photosynthetic organism, *C. limicola* (*C. thiosulfatophilum*) this, rather than the reductive pentose cycle (Calvin cycle), is believed to predominate as the means of CO_2 fixation by this photoautotroph (248). For a detailed discussion of ferredoxin-linked carboxylation reactions, see the review by Buchanan (44).

Aerobic Bacteria

The transport of electrons at a low oxidation-reduction potential in aerobic microorganisms poses problems not encountered in their anaerobic counterparts. Because ferredoxins are highly auto-oxidizable, the aerobe must devise a mechanism to protect the iron-sulfur electron carrier from oxidation by O_2. It is conceivable that O_2 may not be a problem if the reductases that normally oxidize the ferredoxins have a higher affinity for the electrons than does O_2. Aerobic cells, however, have mechanisms to protect other iron-sulfur proteins, such as nitrogenase, from O_2, and such protective mechanisms may also apply to the ferredoxins. In *A. vinelandii*, highly active O_2-consuming respiratory enzymes in the vicinity of nitrogenase are believed to be one way in which the cell protects that enzyme (76) and, presumably, the electron carriers which transport electrons to it.

Ferredoxin and a ferredoxin-dependent reaction in an obligate aerobic bacterium was first reported from *P. putida*, where it participates in the hydroxylation of camphor (63, 136) (the degradation of camphor can provide this organism with a source of both carbon and energy). Analogous to the rubredoxin-dependent ω-hydroxylase system of *P. oleovorans* (Fig. 6), the hydroxylase of *P. putida* requires O_2 and reducing equivalents. The electrons for this reaction are provided by *P. putida* ferredoxin (formerly known as putidaredoxin). The electron flow in this reaction proceeds as follows:

$$\text{NADH} \xrightarrow[\text{reductase}]{\text{NADH-Fd}} \text{Fd} \xrightarrow{e^-} \underset{\text{hydroxylase}}{\text{cytochrome P-450}_{cam}}$$

Nitrogenase is another enzyme which occurs in a few species of aerobic bacteria and which requires reduced ferredoxin as a source of reducing power. *A. vinelandii* (317), *Rhizobium* bacteroids (305, 316), and *M. flavum* (34) have all been shown to use a ferredoxin to drive their respective nitrogenase reactions. Compared with the anaerobes, such as clostridia, there is comparatively little known about these ferredoxin-dependent electron transport processes in aerobic bacteria. Of significance is the fact that respiration is the primary mode of energy metabolism in these organisms, with reduced pyridine nucleotides being the primary source of reductant for biosynthesis. It has long been apparent that the substrates and dehydrogenases that reduce clostridial ferredoxin are not operative in these aerobes.

Reduced pyridine nucleotides have been used to couple electron flow to the nitrogenases of *A. vinelandii* (21) and *Rhizobium* bacteroids (305); however, in both cases the rate was very low. A more active electron donor system included an exogenously added NADP-ferredoxin reductase (from spinach chloroplasts) and a combination of ferredoxin and flavodoxin in addition to the reduced pyridine nucleotide. Although this system is highly artificial, the reactions may be viewed as follows:

$$\text{NAD(P)H} \xrightarrow[\text{reductase}]{\text{NADPH-Fd}}$$

$$\text{Fd, flavodoxin} \xrightarrow{e^-} \text{nitrogenase}$$

The sequence of carriers is not known since both are reduced by the plant reductase. Although these reduced carriers individually donate electrons to nitrogenase if artificially reduced by illuminated chloroplasts (148, 317), their activity is more than additive in the pyridine nucleotide-driven system. Haaker and Veeger (112) have presented an alternative hypothesis for the reduction of *A. vinelandii* nitrogenase, which involves only the flavodoxin and a hypothetical membrane-bound flavodoxin reductase. As in the photosynthetic bacteria, the mechanism of ferredoxin (and flavodoxin) reduction in aerobes remains unknown.

Nitrate reductase in *Azotobacter* is also ferredoxin dependent (279). Previous work has shown that reduced pyridine nucleotide cannot provide reductant for this reaction unless chemically reduced benzyl or methyl viologen is present (110). By using the chloroplast technique (312) to photoreduce *Azotobacter chroococcum* ferredoxin, Guerrero et al. (110) were able to show that the native ferredoxin served as the intermediate electron donor for the reduction of nitrate. Nitrate is reduced by the following reactions:

$$\text{reduced dye} \xrightarrow[\text{light}]{\text{chloroplasts}} Fd_{red} \xrightarrow{e} \text{nitrate reductase} \begin{cases} \rightarrow NO_3^- \\ \rightarrow NO_2^- \end{cases}$$

The final group of aerobic bacteria to be considered is the cyanobacteria. The physiology of the photosynthetic cyanobacteria, however, is completely unlike that of either the aerobic bacteria or the anaerobic photosynthetic bacteria in that they carry out a typical plant-type photosynthesis (Fig. 14) and are capable of respiration. Electrons for the reduction of ferredoxin come from the photooxidation of water and are passed to ferredoxin via Photosystems I and II. Reduced ferredoxin is used to generate reduced pyridine nucleotides for CO_2 fixation and other biosynthetic reactions or is used directly for the reduction of nitrogenase and nitrite reductase and for other reactions.

The fact that the blue-green algae (cyanobacteria) fix N_2 in the dark (165) with rates as high as 50% of those observed in the light indicates that their respiratory system is capable of generating both ATP and reductant in substantial quantity. *Anabaena* oxidizes both isocitrate (253) and glucose 6-phosphate (33) in NADP-linked reactions, and both substrates have been shown to drive ferredoxin-dependent N_2 fixation (33, 253). Other enzymes of the pentose phosphate cycle in blue-green algae are also known to reduce pyridine nucleotides. In addition, pyruvate (62) and H_2 (20) can provide reductant via ferredoxin for the dark fixation of N_2. The reactions that provide reductant by way of ferredoxin for the dark fixation of N_2 by the cyanobacteria are summarized in Fig. 15.

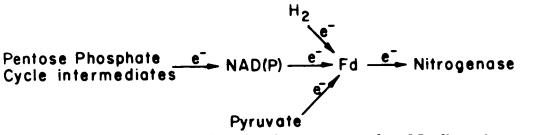


FIG. 15. *Sources of reducing power for N_2 fixation in the dark by cyanobacteria.*

Others

Ferredoxin has been shown to play an important role in both carbon metabolism and energy metabolism of such obligate anaerobes as *Sarcina ventricula*, where it couples to the hydrogenase (260), and *Sarcina maxima*, where it functions in pyruvate-driven NADP reduction (151). In rumen bacteria such as *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*, ferredoxin provides the high-energy electrons for the reductive carboxylation of isobutyrate (10) and succinate (11) for the formation of 2-oxoisovalerate and α-ketoglutarate, which serve as precursors for the biosynthesis of several amino acids in these organisms.

S organism, an anaerobe which forms a symbiotic relationship with a *Methanobacterium* sp.

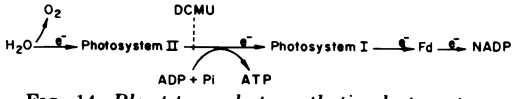
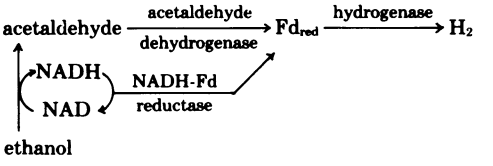


FIG. 14. *Plant-type photosynthetic electron transport to ferredoxin. DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; ADP, adenosine 5'-diphosphate; Pi, inorganic phosphate.*

in a mixed culture formerly known as *Methanobacillus omelianskii* (42), obtains its energy by the fermentation of ethanol to acetate and H_2 (41). Although the alcohol dehydrogenase is NAD linked, the organism contains an NADH-ferredoxin reductase (221, 222) which couples electron flow to hydrogenase. Furthermore, acetaldehyde, the product of ethanol oxidation, is oxidized by a ferredoxin-dependent acetaldehyde dehydrogenase which also couples to hydrogenase, as indicated by the following reaction:



S organism thrives only if the concentration of H_2 is kept at a low level. This appears to have been the function of the methylogenic syntroph, which consumes H_2 as a basis of its energy metabolism (221, 222).

CONCLUDING REMARKS

During the past 15 years our understanding of the metabolism and biochemistry of procaryotes has been greatly advanced due to the findings related to iron-sulfur-containing electron carriers and enzymes. Our knowledge of the ferredoxins now includes detailed information on their distribution among the various bacterial species, their role in specific biochemical processes, and the physical nature of the two basic Fe-S prosthetic groups. In brief, the amount of information now accumulated on ferredoxins is approaching that of the cytochromes, which suggests that future advances in the study of ferredoxins will be of a more esoteric nature. Unlike the ferredoxins with their two basic types of Fe-S clusters, the iron-sulfur-containing enzymes are a much more complicated group of proteins, with many utilizing both the iron-sulfur clusters and metal atoms such as molybdenum or possibly selenium in their electron-carrying processes. Other Fe-S enzymes contain electron-carrying chromophores, such as flavins and hemes, or a combination of these electron-carrying groups and metals, a combination which makes an even more complicated enzyme. It is

obvious from this discussion that both the structure and the mechanism of most of these complex iron-sulfur-containing enzymes will require efforts equal to or greater than those applied to the two (or three) major classes of ferredoxins for their elucidation. With more than a dozen of these multiple-carrier enzymes known to date (Table 3), another generation of researchers will surely find challenging problems to solve in the field of iron-sulfur protein biochemistry.

ACKNOWLEDGMENT

We thank Graham Palmer for his critical reading of the manuscript and constructive comments on it.

ADDENDUM IN PROOF

The Nomenclature Committee of the International Union of Biochemistry in their 1978 recommendations on the nomenclature of iron-sulfur proteins (Eur. J. Biochem. **93**:427–430, 1979) state that when the cluster charge is calculated, the cysteine residues linked to the iron are to be excluded. Therefore, the charge on the Fe-S cluster of an Fe_4 ferredoxin, e.g., *Bacillus polymyxa*, in its normally isolated oxidized state is now (+2) instead of (−2) and that of the reduced *B. polymyxa* ferredoxin is (+1) instead of (−3). The redox transitions of the Fe_4-S_4 cluster of the oxidized minus reduced couple would thus be $+3 \leftrightharpoons +2$ instead of $-2 \leftrightharpoons -3$.

LITERATURE CITED

1. **Adams, M. W. W., and D. O. Hall.** 1977. Isolation of a membrane-bound hydrogenase from *Rhodospirillum rubrum*. Biochem. Biophys. Res. Commun. **77**:730–737.
2. **Adams, M. W. W., S. G. Reeves, D. O. Hall, G. Christou, B. Ridge, and H. N. Rydon.** 1978. Biological activity of synthetic tetranuclear iron-sulfur analogues of the active sites of ferredoxins. Biochem. Biophys. Res. Commun. **79**:1184–1191.
3. **Adman, E. T., L. C. Sieker, and L. H. Jensen.** 1973. The structure of a bacterial ferredoxin. J. Biol. Chem. **248**:3987–3996.
4. **Akagi, J. M.** 1965. The participation of a ferredoxin of *Clostridium nigrificans* in sulfite reduction. Biochem. Biophys. Res. Commun. **21**: 72–77.
5. **Akagi, J. M.** 1967. Electron carriers for the phosphoroclastic reaction of *Desulfovibrio desulfuricans*. J. Biol. Chem. **242**:2478–2483.
6. **Albrecht, S. L., and M. C. W. Evans.** 1973. Measurement of the redox potential of the EPR detectable active center of the molybdenum-iron protein of *Chromatium* nitrogenase. Biochem. Biophys. Res. Commun. **55**:1009–1014.
7. **Aleman, V., and P. Handler.** 1967. Dihydroorotate dehydrogenase. I. General properties. J. Biol. Chem. **242**:4087–4096.
8. **Aleman, V., P. Handler, G. Palmer, and H. Beinert.** 1968. Studies on dihydroorotate dehydrogenase by EPR spectroscopy. II. Electron paramagnetic resonance, optical spectra and titrations. J. Biol. Chem. **243**:2560–2568.
9. **Aleman, V., P. Handler, G. Palmer, and H. Beinert.** 1968. Studies on dihydroorotate dehydrogenase by electron paramagnetic resonance spectroscopy. J. Biol. Chem. **243**:2569–2578.
10. **Allison, M. J., and J. L. Peel.** 1971. The biosynthesis of valine from isobutyrate by *Peptostreptococcus elsdenii* and *Bacteroides ruminicola*. Biochem. J. **121**:431–437.
11. **Allison, M. J., and I. M. Robinson.** 1970. Biosynthesis of α-ketoglutarate by the reductive carboxylation of succinate in *Bacteroides ruminicola*. J. Bacteriol. **104**:50–56.
12. **Andreesen, J. R., and L. G. Ljungdahl.** 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: incorporation of selenium-75 and the effects of selenite, molybdate, and tungstate on the enzyme. J. Bacteriol. **116**:867–873.
13. **Bachofen, R., and D. I. Arnon.** 1966. Crystalline ferredoxin from the photosynthetic bacterium *Chromatium*. Biochim. Biophys. Acta **120**:259–265.
14. **Bachofen, R., B. B. Buchanan, and D. I. Arnon.** 1964. Ferredoxin as a reductant in pyruvate synthesis by a bacterial extract. Proc. Natl. Acad. Sci. U.S.A. **51**:690–694.
15. **Bartsch, R. G.** 1963. Nonheme iron proteins and *Chromatium* iron protein, p. 315–326. *In* H. Gest, A. San Pietro, and L. P. Vernon (ed.), Bacterial photosynthesis. Antioch Press, Yellow Springs, Ohio.
16. **Beinert, H., B. A. C. Ackrell, E. B. Kearney, and T. P. Singer.** 1975. Iron-sulfur components of succinate dehydrogenase: stoichiometry and kinetic behavior in activated preparations. Eur. J. Biochem. **54**:185–194.
17. **Beinert, H., W. Heinen, and G. Palmer.** 1962. Applications of combined low temperature optical and electron paramagnetic resonance spectroscopy to the study of oxidative enzymes. Brookhaven Symp. Biol. **15**:229–265.
18. **Beinert, H., and R. H. Sands.** 1960. Studies on succinic and DPNH dehydrogenase preparations by paramagnetic resonance (EPR) spectroscopy. Biochem. Biophys. Res. Commun. **3**: 41–46.
19. **Bell, G. R., J. LeGall, and H. D. Peck, Jr.** 1974. Evidence for the periplasmic location of hydrogenase in *Desulfovibrio gigas*. J. Bacteriol. **120**:994–997.
20. **Benemann, J. R., and N. M. Weare.** 1974. Nitrogen fixation by *Anabaena cylindrica*. III. Hydrogen-supported nitrogenase activity. Arch. Microbiol. **101**:401–408.
21. **Benemann, J. R., D. C. Yoch, R. C. Valentine, and D. I. Arnon.** 1971. The electron transport system in nitrogen fixation by *Azotobacter*. III. Requirements for NADPH-supported nitrogenase activity. Biochim. Biophys. Acta **226**: 205–212.
22. **Bennett, R., N. Rigopoulos, and R. C. Fuller.** 1964. The pyruvate phosphoroclastic reaction and light dependent nitrogen fixation in bac-

terial photosynthesis. Proc. Natl. Acad. Sci. U.S.A. **52**:762–768.

23. **Bergersen, F. J., and G. L. Turner.** 1973. Kinetic studies of nitrogenase from soya-bean root nodule bacteroids. Biochem. J. **131**:61–75.
24. **Bernhardt, F.-H., N. Erdin, H. Staudinger, and V. Ullrich.** 1973. Interactions of substrates with a purified 4-methoxybenzoate monooxygenase system (o-demethylating) from *Pseudomonas putida*. Eur. J. Biochem. **35**:126–134.
25. **Bernhardt, F.-H., W. Nastainczyk, and V. Seydewitz.** 1977. Kinetic studies on a 4-methoxybenzoate O-demethylase from *Pseudomonas putida*. Eur. J. Biochem. **72**:107–115.
26. **Bernhardt, F.-H., H. Pachowsky, and H. Staudinger.** 1975. A 4-methoxylbenzoate O-demethylase from *Pseudomas putida*. A new type of monooxygenase system. Eur. J. Biochem. **57**:241–256.
27. **Bernhardt, F.-H., and H. H. Ruf.** 1974. A 4-methoxybenzoate monooxygenase system from *Pseudomonas putida*. Circular dichroism studies on the iron-sulfur protein. FEBS Lett. **43**: 53–55.
28. **Bernhardt, F.-H., H. H. Ruf, H. Staudinger, and V. Ullrich.** 1971. Purification of a 4-methoxybenzoate O-demethylase from *Psuedomonas putida*. Hoppe Seyler's Z. Physiol. Chem. **352**:1091–1099.
29. **Blackmore, M. A., and J. R. Quayle.** 1962. Choice between autotrophy and heterotrophy in *Pseudomonas oxalaticus*. Growth on mixed substrates. Biochem. J. **107**:705–713.
30. **Blatmann, P., and J. Rétey.** 1972. Stereospecificity of the dihydroorotate dehydrogenase reaction. Eur. J. Biochem. **30**:130–137.
31. **Bolton, J. R.** 1977. Photosystem I photoreactions, p. 189–202. *In* J. Barber (ed.), Primary processes in photosynthesis. Elsevier, Amsterdam.
32. **Bose, S. K., and H. Gest.** 1962. Electron transport systems in purple bacteria. Hydrogenases and light-stimulated electron transfer reactions in photosynthetic bacteria. Nature (London) **195**:1168–1172.
33. **Bothe, H.** 1972. Relationship between nitrogen-fixation and photosynthesis in blue-green algae, p. 2169–2178. *In* G. Forti, M. Avron, and A. Melandri (ed.), Proceedings of the 2nd International Congress on Photosynthesis. Dr. W. Junk, N.V., The Hague.
34. **Bothe, H., and M. G. Yates.** 1976. The electron transport to nitrogenase in *Mycobacterium flavum*. Arch. Microbiol. **107**:25–31.
35. **Bowen, T. J., F. C. Happold, and B. F. Taylor.** 1966. Studies on adenosine-5'-phosphosulfate reductase from *Thiobacillus denitrificans*. Biochim. Biophys. Acta **118**:566–576.
36. **Bradshaw, W. H., and H. A. Barker.** 1960. Purification and properties of xanthine dehydrogenase from *Clostridium cylindrosporum*. J. Biol. Chem. **235**:3620–3629.
37. **Bramlett, R. N., and H. D. Peck.** 1975. Some physical and kinetic properties of adenylyl sulfate reductase from *Desulfovibrio vulgaris*. J. Biol. Chem. **250**:2979–2986.
38. **Bray, R. C., S. P. Vincent, D. J. Lowe, R. A. Clegg, and P. B. Garland.** 1976. Electron-paramagnetic-resonance studies on the molybdenum of nitrate reductase from *Escherichia coli* K12. Biochem. J. **155**:201–203.
39. **Brintzinger, H., G. Palmer, and R. H. Sands.** 1966. On the ligand field of iron in ferredoxin from spinach chloroplasts and related nonheme iron enzymes. Proc. Natl. Acad. Sci. U.S.A. **55**:397–404.
40. **Bruschi, M., E. C. Hatchikian, J. LeGall, J. J. G. Moura, and A. V. Xavier.** 1976. Purification, characterization and biological activity of three forms of ferredoxin from the sulfate-reducing bacterium *Desulfovibrio gigas*. Biochim. Biophys. Acta **449**:275–284.
41. **Bryant, M. P., B. C. McBride, and R. S. Wolfe.** 1968. Hydrogen-oxidizing methane bacteria. I. Cultivation and methanogenesis. J. Bacteriol. **95**:1118–1123.
42. **Bryant, M. P., E. A. Wolin, M. J. Wolin, and R. S. Wolfe.** 1967. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch. Microbiol. **59**:20–31.
43. **Buchanan, B. B.** 1969. Role of ferredoxin in the synthesis of α-ketobutyrate from propinonyl coenzyme A and carbon dioxide by enzymes from photosynthetic and nonphotosynthetic bacteria. J. Biol. Chem. **244**:4218–4223.
44. **Buchanan, B. B.** 1972. Ferredoxin-linked carboxylation reactions, p. 193–216. *In* P. D. Boyer (ed.), The enzymes, vol. 6, 3rd ed. Academic Press Inc., New York.
45. **Buchanan, B. B., and D. I. Arnon.** 1970. Ferredoxins: chemistry and function in photosynthesis, nitrogen fixation, and fermentative metabolism. Adv. Enzymol. **33**:119–176.
46. **Buchanan, B. B., and R. Bachofen.** 1968. Ferredoxin-dependent reductions of nicotinamide-adenine dinucleotides with hydrogen gas by subcellular preparations from the photosynthetic bacterium, *Chromatium*. Biochim. Biophys. Acta **162**:607–610.
47. **Buchanan, B. B., M. C. W. Evans, and D. I. Arnon.** 1967. Ferredoxin-dependent carbon assimilation in *Rhodospirillum rubrum*. Arch. Mikrobiol. **59**:32–40.
48. **Cammack, R.** 1973. "Super-reduction" of *Chromatium* high-potential iron-sulfur protein in the presence of dimethyl sulphoxide. Biochem. Biophys. Res. Commun. **54**:548–554.
49. **Cammack, R., K. K. Rao, C. P. Bargeron, K. G. Hutson, P. W. Andrew, and L. J. Rogers.** 1977. Midpoint redox potentials of plant and algal ferredoxins. Biochem. J. **168**: 205–209.
50. **Cammack, R., K. K. Rao, D. O. Hall, J. J. G. Moura, A. V. Xavier, M. Bruschi, J. LeGall, A. Deville, and J.-P. Gayda.** 1977. Spectroscopic studies on the oxidation-reduction properties of three forms of ferredoxin from *Desulfovibrio gigas*. Biochim. Biophys. Acta **490**: 311–321.

51. **Cammack, R., E. Tel-Or, and W. D. P. Stewart.** 1976. EPR spectra of Photosystem I constituents in heterocyst preparations from *Anabaena cylindrica*. FEBS Lett. **70**:241–244.
52. **Cardenas, J., L. E. Mortenson, and D. C. Yoch.** 1976. Purification and properties of paramagnetic protein from *Clostridium pasteurianum* W5. Biochim. Biophys. Acta **434**:244–257.
53. **Carithers, R. P., D. C. Yoch, and D. I. Arnon.** 1977. Isolation and characterization of bound iron-sulfur proteins from bacterial photosynthetic membranes. II. Succinate dehydrogenase from *Rhodospirillum rubrum* chromatophores. J. Biol. Chem. **252**:7461–7467.
54. **Carter, C. W., Jr., S. T. Freer, N. H. Xuong, R. A. Alden, and J. Kraut.** 1971. Structure of the iron-sulfur cluster in the *Chromatium* iron protein at 2.25 Å resolution. Cold Spring Harbor Symp. Quant. Biol. **36**:381–385.
55. **Carter, C. W., Jr., J. Kraut, S. T. Freer, R. A. Alden, L. C. Sieker, E. Adman, and L. H. Jensen.** 1972. A comparison of Fe_4S_4* clusters in high-potential iron protein and in ferredoxin. Proc. Natl. Acad. Sci. U.S.A. **69**:3526–3529.
56. **Cato, E. P., W. E. C. Moore, and L. V. Holdeman.** 1968. *Clostridium oroticum* comb. nov., amended description. Int. J. Syst. Bacteriol. **17**:9–13.
57. **Chen, J.-S., and D. K. Blanchard.** 1978. Isolation and properties of a unidirectional H_2-oxidizing hydrogenase from the strictly anaerobic N_2-fixing bacterium *Clostridium pasteurianum* W5. Biochem. Biophys. Res. Commun. **84**:1144–1150.
58. **Chen, J.-S., and L. E. Mortenson.** 1974. Purification and properties of hydrogenase from *Clostridium pasteurianum* W5. Biochim. Biophys. Acta **371**:283–298.
59. **Chen, J.-S., L. E. Mortenson, and G. Palmer.** 1976. The iron-sulfur centers and the function of hydrogenase from *Clostridium pasteurianum*, p. 68–82. *In* K. T. Yasunobu, H. F. Mower, and O. Hayaishi (ed.), Iron and copper proteins. Plenum Publishing Corp., New York.
60. **Chiba, S., and M. Ishimoto.** 1973. Ferredoxin-linked nitrate reductase from *Clostridium perfringens*. J. Biochem. **73**:1315–1318.
61. **Clegg, R. A.** 1976. Purification and some properties of nitrate reductase (EC 1.7.9.9.4) from *Escherchia coli* K12. Biochem. J. **153**:533–541.
62. **Codd, G. A., P. Rowell, and W. D. P. Stewart.** 1974. Pyruvate and nitrogenase activity in cell-free extracts of the blue-green alga *Anabaena cylindrica*. Biochem. Biophys. Res. Commun. **61**:424–431.
63. **Cushman, D. W., R. L. Tsai, and I. C. Gunsalus.** 1967. The ferroprotein component of a hydroxylase. Biochem. Biophys. Res. Commun. **26**:577–583.
64. **Dalton, H., J. A. Morris, M. A. Ward, and L. E. Mortenson.** 1971. Purification and properties of molybdoferredoxin, a component of nitrogenase from *Clostridium pasteurianum*. Biochemistry **10**:2066–2072.
65. **Dalton, H. D., D. J. Lowe, R. Pawlik, and R. C. Brays.** 1976. Studies by EPR spectroscopy on the mechanism of action of xanthine dehydrogenase from *Veillonella alcalescens*. Biochem. J. **153**:287–295.
66. **Dancey, G. F., A. E. Levine, and B. M. Shapiro.** 1976. The NADH dehydrogenase of the respiratory chain of *Escherichia coli*. J. Biol. Chem. **251**:5911–5920.
67. **Dancey, G. F., and B. M. Shapiro.** 1976. The NADH dehydrogenase of the respiratory chain of *Escherichia coli*. II. Kinetics of the purified enzyme and the effects of antibodies elicited against it on membrane-bound and free enzyme. J. Biol. Chem. **251**:5921–5928.
68. **Davis, K. A., Y. Hatefi, I. P. Crawford, and H. Baltscheffsky.** 1977. Purification, molecular weight and amino acid composition of the subunits of *Rhodospirillum rubrum* succinate dehydrogenase. Arch. Biochem. Biophys. **180**:459–467.
69. **DerVartanian, D. V.** 1972. NADH dehydrogenases from *Azotobacter vinelandii*. Z. Naturforsch. **276**:1082–1084.
70. **DerVartanian, D. V., and R. Bramlett.** 1970. Electron paramagnetic resonance studies of ^{95}Mo-enriched NADH dehydrogenase isolated from iron-deficient *Azotobacter vinelandii*. Biochim. Biophys. Acta **220**:443–448.
71. **DerVartanian, D. V., and P. Forget.** 1975. The bacterial nitrate reductase. EPR studies on the enzyme A of *Escherichia coli* K12. Biochim. Biophys. Acta **379**:74–80.
72. **DerVartanian, D. V., Y. I. Shethna, and H. Beinert.** 1969. Purification and properties of two iron-sulfur proteins from *Azotobacter vinelandii*. Biochim. Biophys. Acta **194**:548–563.
73. **Dilworth, M. J.** 1966. Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. Biochim. Biophys. Acta **127**:285–294.
74. **Dixon, R. O. D.** 1972. Hydrogenase in legume root nodule bacteroids: occurrence and properties. Arch. Microbiol. **85**:193–201.
75. **Dixon, R. O. D.** 1976. Hydrogenase and efficiency of nitrogen fixation in aerobes. Nature (London) **262**:173.
76. **Drozd, J., and J. R. Postgate.** 1970. Effects of oxygen on acetylene reduction, cytochrome content and respiratory activity of *Azotobacter chroococcum*. J. Gen. Microbiol. **63**:63–73.
77. **Durham, W. R., G. Palmer, R. H. Sands, and A. J. Bearden.** 1971. On the structure of the iron-sulfur complex in the two-iron ferredoxins. Biochim. Biophys. Acta **253**:373–384.
78. **Dutton, P. L., and J. S. Leigh.** 1973. Electron spin resonance characterization of *Chromatium* D hemes, non-heme irons and the components involved in primary photochemistry. Biochim. Biophys. Acta **314**:178–190.
79. **Dutton, P. L., and D. F. Wilson.** 1974. Redox potentiometry in mitochondrial and photosynthetic bioenergetics. Biochim. Biophys. Acta **346**:165–212.
80. **Eady, R. R.** 1973. Nitrogenase of *Klebsiella*

pneumoniae. Interaction of the component proteins studied by ultracentrifugation. Biochem. J. **135**:531–535.

81. **Eady, R. R., C. Kennedy, B. E. Smith, R. N. F. Thornley, G. Yates, and J. R. Postgate.** 1975. Nitrogenase in *Azotobacter chroococcum* and *Klebsiella pneumoniae*. Biochem. Soc. Trans. **3**:488–492.
82. **Eady, R. R., B. E. Smith, K. A. Cook, and J. R. Postgate.** 1972. Nitrogenase of *Klebsiella pneumoniae*. Purification and properties of the component proteins. Biochem. J. **128**:655–675.
83. **Edmondson, D., V. Massey, G. Palmer, L. M. Beacham, and G. B. Elion.** 1972. The resolution of active and inactive xanthine oxidase by affinity chromatography. J. Biol. Chem. **247**:1597–1604.
84. **Elmerich, C., and J.-P. Aubert.** 1971. Synthesis of glutamate by a glutamine: 2-oxo-glutarate amidotransferase (NADP oxidoreductase) in *Bacillus megaterium*. Biochem. Biophys. Res. Commun. **42**:371–376.
85. **Ely, B., A. B. C. Amarasinghe, and R. A. Bender.** 1978. Ammonia assimilation and glutamate formation in *Caulobacter crescentus*. J. Bacteriol. **133**:225–230.
86. **Enoch, H. G., and R. L. Lester.** 1974. The role of a novel cytochrome *b*-containing nitrate reductase and quinone in the *in vitro* reconstitution of formate-nitrate reductase activity of *Escherichia coli*. Biochem. Biophys. Res. Commun. **61**:1234–1241.
87. **Enoch, H. G., and R. L. Lester.** 1975. The purification and properties of formate dehydrogenase and nitrate reductase from *Escherichia coli*. J. Biol. Chem. **250**:6693–6705.
88. **Erbes, D. L., R. H. Burris, and W. H. Orme-Johnson.** 1975. On the iron-sulfur cluster of hydrogenase from *Clostridium pasteurianum* W5. Proc. Natl. Acad. Sci. U.S.A. **72**:4795–4799.
89. **Evans, E. H., R. Cammack, and W. C. W. Evans.** 1976. Properties of the primary electron acceptor of Photosystem I in the blue-green alga *Chlorogloea fritschii*. Biochem. Biophys. Res. Commun. **68**:1212–1218.
90. **Evans, M. C. W.** 1968. Ferredoxin dependent synthesis of α-ketoglutarate and pyruvate by extracts of the green photosynthetic bacterium *Chloropseudomonas ethylicum*. Biochem. Biophys. Res. Commun. **33**:146–150.
91. **Evans, M. C. W.** 1977. Electron paramagnetic-resonance studies in photosynthesis, p. 433–464. *In* J. Barber (ed.), Primary processes in photosynthesis. Elsevier, Amsterdam.
92. **Evans, M. C. W., and S. L. Albrecht.** 1974. Determination of the applied redox potential required for substrate reduction by *Chromatium* nitrogenase. Biochem. Biophys. Res. Commun. **61**:1187–1192.
93. **Evans, M. C. W., and B. B. Buchanan.** 1965. Photoreduction of ferredoxin and its use in carbon dioxide fixation by a subcellular system from a photosynthetic bacterium. Proc. Natl. Acad. Sci. U.S.A. **53**:1420–1425.
94. **Evans, M. C. W., A. V. Lord, and S. G. Reeves.** 1974. The detection and characterization by electron-paramagnetic resonance spectroscopy of iron-sulfur proteins and other electron transport components in chromatophores from the purple bacterium *Chromatium*. Biochem. J. **138**:177–183.
95. **Evans, M. C. W., S. G. Reeves, and A. Telfer.** 1973. The detection of a bound ferredoxin in the photosynthetic lamellae of blue-green algae and other oxygen evolving photosynthetic organisms. Biochem. Biophys. Res. Commun. **51**: 593–596.
96. **Evans, M. C. W., A. Telfer, and R. V. Smith.** 1973. The purification and some properties of the molybdenum-iron protein of *Chromatium* nitrogenase. Biochim. Biophys. Acta **310**:344–352.
97. **Faeder, E. J., P. S. Davis, and L. M. Siegel.** 1974. Reduced nicotinamide adenine dinucleotide phosphate sulfite reductase of enterobacteria. Studies with the *Escherichia coli* hemoflavoprotein depleted of flavin mononucleotide: distinct roles for the flavin adenine dinucleotide and flavin mononucleotide prosthetic groups in catalysis. J. Biol. Chem. **249**:1599–1609.
98. **Feigenblum, E., and A. I. Krasna.** 1970. Solubilization and properties of the hydrogenase of *Chromatium*. Biochim. Biophys. Acta **198**: 157–164.
99. **Forget, P.** 1974. The bacterial nitrate reductases. Solubilization, purification and properties of enzyme A of *Escherichia coli* K12. Eur. J. Biochem. **42**:325–332.
100. **Forget, P., and D. V. DerVartanian.** 1972. The bacterial nitrate reductase: EPR studies on nitrate reductase A from *Micrococcus denitrificans*. Biochim. Biophys. Acta **256**:600–606.
101. **Friedmann, H. C., and B. Vennesland.** 1960. Crystalline dihydroorotic dehydrogenase. J. Biol. Chem. **235**:1526–1532.
102. **Fritz, J., R. Anderson, J. Fee, G. Palmer, R. H. Sands, J. C. Tsibris, I. C. Gunsalus, W. H. Orme-Johnson, and H. Beinert.** 1971. The iron electron-nuclear double resonance (ENDOR) of two-iron ferredoxins from spinach, parsley, pig adrenal cortex and *Pseudomonas putida*. Biochim. Biophys. Acta **253**:110–133.
103. **Geary, L. E., and A. Meister.** 1977. On the mechanism of glutamine-dependent reductive amination of α-ketoglutarate catalyzed by glutamate synthase. J. Biol. Chem. **252**:3501–3508.
104. **Gehring, U., and D. I. Arnon.** 1971. Ferredoxin-dependent phenylpyruvate synthesis by cell-free preparations of photosynthetic bacteria. J. Biol. Chem. **246**:4518–4522.
105. **Gillum, W. O., L. E. Mortenson, J.-S. Chen, and R. H. Holm.** 1977. Quantitative extrusions of the $Fe_4S_4^*$ cores of the active sites of ferredoxins and hydrogenase of *Clostridium pasteurianum*. J. Am. Chem. Soc. **99**:584–595.
106. **Gitlitz, P. H., and A. I. Krasna.** 1975. Structural

and catalytic properties of hydrogenase from *Chromatium*. Biochemistry **14**:2561–2568.

107. **Gogotov, I. N., and T. V. Laurinavichene.** 1975. The role of ferredoxin in the hydrogen metabolism in *Rhodospirillum rubrum*. Mikrobiologiya **44**:581–586.
108. **Gogotov, I. N., N. A. Zorin, L. T. Serebriakova, and E. N. Kondratieva.** 1978. The properties of hydrogenase from *Thiocapsa roseopersicina*. Biochim. Biophys. Acta **523**: 335–343.
109. **Gray, C. T., and H. Gest.** 1965. Biological formation of molecular hydrogen. A "hydrogen valve" facilitates regulation of anaerobic energy metabolism in many organisms. Science **148**:186–192.
110. **Guerrero, M. G., J. M. Vega, E. Leadbetter, and M. Losada.** 1973. Preparation and characterization of a soluble nitrate reductase from *Azotobacter chroococcum*. Arch. Mikrobiol. **91**:287–304.
111. **Gutman, M., A. Schejter, and Y. Avi-Dor.** 1968. The preparation and properties of the membranal DPNH dehydrogenase from *Escherichia coli*. Biochim. Biophys. Acta **162**:506–517.
112. **Haaker, H., and C. Veeger.** 1977. Involvement of the cytoplasmic membrane in nitrogen fixation by *Azotobacter vinelandii*. Eur. J. Biochem. **77**:1–10.
113. **Hall, D. O., K. K. Rao, and R. Cammack.** 1975. The iron-sulfur proteins: structure, function and evolution of a ubiquitous group of proteins. Sci. Prog. (London) **62**:285–317.
114. **Hardy, R. W. F., and R. C. Burns.** 1968. Biological nitrogen fixation. Annu. Rev. Biochem. **37**:331–358.
115. **Hardy, R. W. F., F. Knight, Jr., C. C. McDonald, and A. J. D'Eustachio.** 1965. Paramagnetic protein from nitrogen-fixing extracts of *Clostridium pasteurianum*, p. 275–282. *In* A. San Pietro (ed.), Non-heme iron proteins: role of energy conversion. Antioch Press, Yellow Springs, Ohio.
116. **Hatchikian, E. C., M. Bruschi, and J. LeGall.** 1978. Characterization of the periplasmic hydrogenase from *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. **82**:451–461.
117. **Hatefi, Y., K. A. Davis, H. Baltscheffsky, M. Baltscheffsky, and B. C. Johansson.** 1972. Isolation and properties of succinate dehydrogenase from *Rhodospirillum rubrum*. Arch. Biochem. Biophys. **152**:613–618.
118. **Herskovitz, T., B. A. Averill, K. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weihers.** 1972. Structure and properties of a synthetic analogue of bacterial iron-sulfur proteins. Proc. Natl. Acad. Sci. U.S.A. **69**:2437–2441.
119. **Higuti, T., T. Shiga, T. Kakuno, and T. Horio.** 1974. Studies on ESR spectra of chromatophores from *Rhodospirillum rubrum*. J. Biochem. **75**:1363–1371.
120. **Hill, C. L., D. J. Steenkamp, R. H. Holm, and T. P. Singer.** 1977. Identification of the iron-sulfur center in trimethylamine dehydrogenase. Proc. Natl. Acad. Sci. U.S.A. **74**:547–551.
121. **Holm, R. H., and J. A. Ibers.** 1977. Synthetic analogues of the active sites of iron-sulfur proteins, p. 205–281. *In* W. Lovenberg (ed.), Iron-sulfur proteins, vol. 3. Academic Press Inc., New York.
122. **Höpner, T., U. Muller, U. Ruschig, and P. Willnow.** 1975. Formate dehydrogenase: preparation and examination of FMN, deflavo, FAD and riboflavin enzymes, p. 582–592. *In* T. P. Singer (ed.), Flavins and flavoproteins, Proceedings of the Fifth International Symposium. Elsevier Scientific Publishing Co., Amsterdam.
123. **Höpner, T., and A. Trautwein.** 1972. Some properties of formate dehydrogenase. Z. Naturforsh. **27b**:1075–1076.
124. **Horio, K.** 1961. Electron-transporting components participating in nitrate and oxygen respirations from a halotolerant *Micrococcus*. J. Biochem. **50**:481–485.
125. **Howard, J. B., T. Lorsbach, and L. Que.** 1976. Iron-sulfur clusters and cysteine distribution in a ferredoxin from *Azotobacter vinelandii*. Biochem. Biophys. Res. Commun. **70**:582–588.
126. **Huang, T. C., W. G. Zumft, and L. E. Mortenson.** 1973. Structure of the molybdoferredoxin complex from *Clostridium pasteurianum* and isolation of its subunits. J. Bacteriol. **113**:884–890.
127. **Hutber, G. N., A. J. Smith, and L. J. Rogers.** 1978. Comparative biological activities of two ferredoxins and a flavodoxin from the cyanobacterium *Nostoc* strain MAC. FEMS Microbiol. Lett. **4**:11–14.
128. **Hutson, K. G., L. J. Rogers, B. G. Haslett, D. Boulter, and R. Cammack.** 1978. Comparative studies on two ferredoxins from the cyanobacterium *Nostoc* strain MAC. Biochem. J **172**:465–477.
129. **Ingledew, W. J., and R. C. Prince.** 1977. Thermodynamic resolution of the iron-sulfur centers of the succinic dehydrogenase of *Rhodopseudomonas sphaeroides*. Arch. Biochem. Biophys. **178**:303–307.
130. **Israel, D. W., R. L. Howard, H. J. Evans, and S. A. Russell.** 1974. Purification and characterization of the molybdenum-iron protein component of nitrogenase from soybean nodule bacteroids. J. Biol. Chem. **249**:500–508.
131. **Johnson, P. W., and E. Canale-Parola.** 1973. Properties of rubredoxin and ferredoxin from isolated spirochetes. Arch. Mikrobiol. **89**:341–353.
132. **Jungermann, K., H. Kirchniawy, and R. K. Thauer.** 1970. Ferredoxin-dependent CO_2 reduction to formate in *Clostridium pasteurianum*. Biochem. Biophys. Res. Commun. **41**: 682–689.
133. **Jungermann, K., E. Rupprecht, C. Ohrloff, R. Thauer, and K. Decker.** 1971. Regulation of the reduced nicotinamide adenine dinucleotide-ferredoxin reductase system in *Clostridium kluyveri*. J. Biol. Chem. **246**:960–963.
134. **Jungermann, K., R. K. Thauer, G. Leimenstoll, and K. Decker.** 1973. Function of re-

duced pyridine nucleotide-ferrodoxin oxidoreductase in saccharolytic clostridia. Biochim. Biophys. Acta **305**:268–280.

135. **Kakuno, T., N. O. Kaplan, and M. D. Kamen.** 1977. *Chromatium* hydrogenase. Proc. Natl. Acad. Sci. U.S.A. **74**:861–863.
136. **Katagiri, M., B. N. Ganguli, and I. C. Gunsalus.** 1968. A soluble cytochrome P-450 functional in methylene hydroxylation. J. Biol. Chem. **243**:3543–3545.
137. **Ke, B., W. A. Bulen, E. R. Shaw, and R. E. Breeze.** 1974. Determination of oxidation-reduction potentials by spectro-polarimetric titration: application to several iron-sulfur proteins. Arch. Biochem. Biophys. **162**:301–309.
138. **Kearney, J. J., and R. D. Sagers.** 1972. Formate dehydrogenase from *Clostridium acidiurici*. J. Bacteriol. **109**:152–161.
139. **Keister, D. L., and N. J. Yike.** 1967. Energy-linked reactions in photosynthetic bacteria. I. Succinate-linked ATP-driven NAD^+ reduction by *Rhodospirillum rubrum* chromatophores. Arch. Biochem. Biophys. **121**:415–422.
140. **Kemp, M. B., B. A. Haddock, and P. B. Garland.** 1975. Synthesis and sidedness of membrane-bound respiratory nitrate reductase (EC 1.7.99.4) in *Escherichia coli* lacking cytochromes. Biochem. J. **148**:329–333.
141. **Kerscher, L., and D. Oesterhelt.** 1977. Ferredoxin is the coenzyme of α-ketoacid oxidoreductases in *Halobacterium halobium*. FEBS Lett. **83**:197–201.
142. **Kerscher, L., D. Oesterhelt, R. Cammack, and D. O. Hall.** 1976. A new plant-type ferredoxin from halobacteria. Eur. J. Biochem. **71**: 101–107.
143. **Kim, I. C., and P. D. Bragg.** 1971. Some properties of the succinate dehydrogenase of *Escherichia coli*. Can. J. Biochem. **49**:1098–1104.
144. **Kimura, T., J. Hauber, and T. P. Singer.** 1967. Studies on succinate dehydrogenase. XIII. Reversible activation of the mammalian enzyme. J. Biol. Chem. **242**:4987–4993.
145. **Kleiner, D., and C. H. Chen.** 1974. Physical and chemical properties of the nitrogenase proteins from *Azotobacter vinelandii*. Arch. Microbiol. **98**:93–100.
146. **Knaff, D. B., and R. Malkin.** 1976. Iron-sulfur proteins of the green photosynthetic bacterium *Chlorobium*. Biochim. Biophys. Acta **430**:244–252.
147. **Knoell, H.-E., and J. Knappe.** 1974. *Escherichia coli* ferredoxin, an iron-sulfur protein of the adrenodoxin type. Eur. J. Biochem. **50**: 245–252.
148. **Koch, B., P. Wong, S. A. Russell, R. Howard, and H. J. Evans.** 1970. Purification and some properties of a non-heme iron protein from the bacteroids of soya-bean (*Glycine max* Merr) nodules. Biochem. J. **118**:773–781.
149. **Kröger, A.** 1978. Fumarate as terminal acceptor of phosphorylative electron transport. Biochim. Biophys. Acta **505**:129–145.
150. **Kröger, A., and A. Innerhofer.** 1976. The function of menaquinone, covalently bound FAD and iron-sulfur protein in the electron transport from formate to fumarate in *Vibrio succinogenes*. Eur. J. Biochem. **69**:487–495.
151. **Kupfer, D. G., and E. Canale-Parola.** 1967. Pyruvate metabolism in *Sarcina maxima*. J. Bacteriol. **94**:984–990.
152. **Laishley, E. J., J. Travis, and H. D. Peck, Jr.** 1969. Amino acid composition of ferredoxin and rubredoxin isolated from *Desulfovibrio gigas*. J. Bacteriol. **98**:302–303.
153. **Lam, Y., and D. J. D. Nicholas.** 1969. A nitrate reductase from *Micrococcus denitrificans*. Biochim. Biophys. Acta **178**:225–234.
154. **Lara, F. J. S.** 1959. The succinic dehydrogenase of *Propionibacterium pentosaceum*. Biochim. Biophys. Acta **53**:565–567.
155. **Lee, J.-P., J. LeGall, and H. D. Peck, Jr.** 1973. Isolation of assimilatory- and dissimilatory-type sulfite reductase from *Desulfovibrio vulgaris*. J. Bacteriol. **115**:529–542.
156. **Lee, J. P., and H. D. Peck, Jr.** 1971. Purification of the enzyme reducing bisulfite to trithionite from *Desulfovibrio gigas* and its identification as desulfoviridin. Biochem. Biophys. Res. Commun. **45**:583–589.
157. **LeGall, J., and N. Dragoni.** 1966. Dependence of sulfite reduction on a crystallized ferredoxin from *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. **23**:145–149.
158. **Li, L. F., L. Ljungdahl, and H. G. Wood.** 1966. Properties of nicotinamide adenine dinucleotide phosphate-dependent formate dehydrogenase from *Clostridium thermoaceticum*. J. Bacteriol. **92**:405–412.
159. **Linnane, A. W., and C. W. Wrigley.** 1963. Fragmentation of the electron transport chain of *Escherichia coli*. Preparation of a soluble formate dehydrogenase-cytochrome b_1 complex. Biochim. Biophys. Acta **77**:408–418.
160. **Ljones, T., and R. H. Burris.** 1972. ATP hydrolysis and electron transfer in the nitrogenase reaction with different combinations of iron-protein and the molybdenum-iron protein. Biochim. Biophys. Acta **275**:93–101.
161. **Ljones, T., and R. H. Burris.** 1978. Evidence for one-electron transfer by the Fe protein of nitrogenase. Biochem. Biophys. Res. Commun. **80**:22–25.
162. **Lovenberg, W.** 1974. Ferredoxin and rubredoxin in microbial iron metabolism, p. 161–185. *In* J. B. Neilands (ed.), Microbial iron metabolism. Academic Press Inc., New York.
163. **Lovenberg, W. (ed.).** 1977. Iron-sulfur proteins, vol. 1, 2, and 3. Academic Press Inc., New York.
164. **Lund, K., and J. A. DeMoss.** 1976. Association-dissociation behavior and subunit structure of heat-treated nitrate reductase from *Escherichia coli*. J. Biol. Chem. **251**:2207–2216.
165. **Lyne, R. A., and W. D. P. Stewart.** 1973. Emerson enhancement of carbon dioxide fixation but not of acetylene reduction (nitrogenase activity) in *Anabaena cylindrica*. Planta **109**:27–38.

166. **MacGregor, C. H.** 1975. Solubilization of *Escherichia coli* nitrate reductase by a membrane-bound protease. J. Bacteriol. **121**:1102–1110.
167. **MacGregor, C. H.** 1975. Anaerobic cytochrome b_1 in *Escherichia coli*. Association and regulation of nitrate reductase. J. Bacteriol. **121**: 1111–1116.
168. **MacGregor, C. H., C. A. Schnaitman, D. E. Normansell, and M. G. Hodgins.** 1974. Purification and properties of nitrate reductase from *Escherichia coli* K12. J. Biol. Chem. **249**: 5321–5327.
169. **Magasanik, B., M. Prival, J. Brenchley, B. Tyler, A. Deleo, S. Streicher, R. Bender, and C. Paris.** 1974. Glutamine synthetase as a regulator of enzyme synthesis. Curr. Top. Cell. Regul. **8**:119–138.
170. **Malkin, R., and A. J. Bearden.** 1978. Membrane bound iron-sulfur centers in photosynthetic systems. Biochim. Biophys. Acta **505**: 147–181.
171. **Massey, V., and D. Edmondson.** 1970. On the mechanism of inactivation of xanthine oxidase by cyanide. J. Biol. Chem. **245**:6595–6598.
172. **Mathews, R., S. Charlton, R. H. Sands, and G. Palmer.** 1974. On the nature of the spin coupling between the iron-sulfur clusters in the eight-iron ferredoxins. J. Biol. Chem. **249**: 4326–4328.
173. **Mayerk, J. J., R. B. Frankel, R. H. Holm, J. A. Ibers, W. D. Phillips, and J. F. Weiher.** 1973. Synthetic analogs of the active sites of iron-sulfur proteins. Structure and properties of {o-xylyldithiolato-μ_2-sulfidoferrate (III)}, an analog of the 2Fe-2S proteins. Proc. Natl. Acad. Sci. U.S.A. **70**:2429–2433.
174. **McIntosh, A. R., M. Chu, and J. R. Bolton.** 1975. Flash photolysis electron spin resonance studies of the electron acceptor species at low temperatures in Photosystem I of spinach subchloroplast particles. Biochim. Biophys. Acta **376**:308–314.
175. **McKenna, E. J., and M. J. Coon.** 1970. Enzymatic ω-oxidation. IV. Purification and properties of the ω-hydroxylase of *Pseudomonas oleovorans*. J. Biol. Chem. **245**:3882–3889.
176. **McKenna, E. J., and R. E. Kallio.** 1965. The biology of hydrocarbons. Annu. Rev. Microbiol. **19**:183–208.
177. **Michaels, G. B., J. T. Davidson, and H. D. Peck.** 1971. Studies on the mechanism of adenyl sulfate reductase from the sulfate reducing bacteria, *Desulfovibrio vulgaris*, p. 555–580. *In* H. Kamin (ed.), Flavins and Flavoproteins, Third International Symposium. University Park Press, Baltimore.
178. **Miller, R. E., and E. R. Stadtman.** 1972. Glutamate synthase from *Escherichia coli*, an iron-sulfide flavoprotein. J. Biol. Chem. **247**: 7407–7419.
179. **Miller, R. W., and V. Massey.** 1965. Dihydroorotic dehydrogenase. I. Some properties of the enzyme. J. Biol. Chem. **240**:1453–1456.
180. **Mortenson, L. E., and J. S. Chen.** 1974. Hydrogenase, p. 231–282. *In* J. B. Nielands (ed.), Microbial iron metabolism. Academic Press Inc., New York.
181. **Mortenson, L. E., J. A. Morris, and D. Y. Jeng.** 1967. Purification, metal composition and properties of molybdoferredoxin and azoferredoxin, two of the components of the nitrogen-fixing system of *Clostridium pasteurianum*. Biochim. Biophys. Acta **141**:516–522.
182. **Mortenson, L. E., R. C. Valentine, and J. E. Carnahan.** 1963. Ferredoxin in the phosphoroclastic reaction of pyruvic acid and its relation to nitrogen fixation in *Clostridium pasteurianum*. J. Biol. Chem. **238**:794–800.
183. **Mortenson, L. E., W. G. Zumft, T. C. Huang, and G. Palmer.** 1973. The structure and function of nitrogenase of *Clostrium pasteurianum*: electron-paramagnetic-resonance studies. Biochem. Soc. Trans. **1**:35–37.
184. **Mortenson, L. E., W. G. Zumft, and G. Palmer.** 1973. Electron paramagnetic resonance studies of nitrogenase. III. Function of magnesium adenosine 5′-triphosphate and adenosine 5′-diphosphate in catalysis by nitrogenase. Biochim. Biophys. Acta **292**:422–435.
185. **Moura, J. J. G., A. V. Xavier, M. Bruschi, J. LeGall, D. O. Hall, and R. Cammack.** 1976. A molybdenum-containing iron-sulfur protein from *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. **72**:782–789.
186. **Moura, J. J. G., A. V. Xavier, D. J. Cookson, G. R. Moore, R. J. P. Williams, M. Bruschi, and J. LeGall.** 1977. Redox states of cytochrome c_3 in the absence and presence of ferredoxin. FEBS Lett. **81**:275–280.
187. **Moura, J. J. G., A. V. Xavier, E. C. Hatchikian, and J. LeGall.** 1978. Structural control of the redox potentials and of the physiological activity by oligomerization of ferredoxin. FEBS Lett. **89**:177–179.
188. **Mullinger, R. N., R. Cammack, K. K. Rao, D. O. Hall, D. P. E. Dickson, C. E. Johnson, J. D. Rush, and A. Simopoulos.** 1975. Physicochemical characterization of the four-iron-four-sulfide ferredoxin from *Bacillus stearothermophilus*. Biochem. J. **151**:75–83.
189. **Munck, E., H. Rhodes, W. H. Orme-Johnson, L. C. Davis, W. J. Brill, and V. K. Shah.** 1975. Nitrogenase. VIII. Mössbauer and EPR spectroscopy. The FeMo protein component from *Azobacter vinelandii* OP. Biochim. Biophys. Acta **400**:32–53.
190. **Murphy, M. J., and L. M. Siegel.** 1973. Siroheme and sirohydrochlorin. The basis for a new type of prophyrin-related prosthetic group common to both assimilatory and dissimilatory sulfite reductases. J. Biol. Chem. **248**:6911–6919.
191. **Nakos, G., and L. E. Mortenson.** 1971. Purification and properties of hydrogenase, an iron-sulfur protein from *Clostridium pasteurianum* W5. Biochim. Biophys. Acta **227**:576–583.
192. **Nakos, G., and L. E. Mortenson.** 1971. Structural properties of hydrogenase from *Clostridium pasteurianum* W5. Biochemistry **10**:2442–2449.

193. **Nagatani, H., M. Shimizu, and R. C. Valentine.** 1971. The mechanism of ammonia assimilation in nitrogen fixing bacteria. Arch. Mikrobiol. **79**:164-175.
194. **Neilands, J. B. (ed.).** 1974. Microbial iron metabolism, a comprehensive treatise. Academic Press Inc., New York.
195. **Nelson, C. A., and P. Handler.** 1968. Preparation of bovine xanthine oxidase and the subunit structures of some iron flavoproteins. J. Biol. Chem. **243**:5368-5373.
196. **Ohnishi, T., J. Lim, D. B. Winter, and T. E. King.** 1976. Thermodynamic and EPR characteristics of a HiPIP-type iron-sulfur center in the succinate dehydrogenase of the respiratory chain. J. Biol. Chem. **251**:2105-2109.
197. **Ohnishi, T., J. C. Salerno, D. B. Winter, J. Lim, C. A. Yu, L. Yu, and T. E. King.** 1976. Thermodynamic and EPR characteristics of two ferredoxin-type iron-sulfur centers in the succinate-ubiquinone reductase segment of the respiratory chain. J. Biol. Chem. **251**:2094-2104.
198. **Olson, J. S., D. P. Ballou, G. Palmer, and V. Massey.** 1974. The mechanism of action of xanthine oxidase. J. Biol. Chem. **249**:4363-4382.
199. **Orme-Johnson, W. H.** 1973. Iron-sulfur proteins: structure and function. Annu. Rev. Biochem. **42**:159-204.
200. **Orme-Johnson, N. R., R. E. Hansen, and H. Beinert.** 1974. Electron paramagnetic resonance-detectable electron acceptors in beef heart mitochondria. J. Biol. Chem. **249**:1922-1927.
201. **Orme-Johnson, W. H., and L. C. Davis.** 1977. Current topics and problems in the enzymology of nitrogenase, p. 15-60. *In* W. Lovenberg (ed.), Iron-sulfur proteins. Academic Press Inc., New York.
202. **Orme-Johnson, W. H., N. A. Stombaugh, and R. H. Burris.** 1972. An iron-sulfur protein (ISP) from *Bacillus polymyxa* with properties intermediate between those of plant and bacterial-type ferredoxin. Fed. Proc. **31**:448.
203. **Orme-Johnson, W. J., W. D. Hamilton, T. Ljones, M. W. Tso, R. H. Burris, V. K. Shah, and W. J. Brill.** 1972. Electron paramagnetic resonance signals of nitrogenase and nitrogenase components from *Clostridium pasteurianum* and *Azotobacter vinelandii*. Proc. Natl. Acad. Sci. U.S.A. **69**:3142-3145.
204. **Packer, L., and W. Vischniac.** 1955. The specificity of a diphosphopyridine nucleotide-linked hydrogenase. Biochim. Biophys. Acta **17**:153-154.
205. **Palmer, G., J. S. Multani, W. C. Cretney, W. G. Zumft, and L. E. Mortenson.** 1972. EPR studies on nitrogenase. I. The properties of molybdoferredoxin and azoferredoxin. Arch. Biochem. Biophys. **153**:325-332.
206. **Palmer, G., R. H. Sands, and L. E. Mortenson.** 1966. Electron paramagnetic resonance studies on the ferredoxin from *Clostridium pasteurianum*. Biochem. Biophys. Res. Commun. **23**:357-367.
207. **Papa, S., M. Lorusso, and F. Guerrieri.** 1975. Mechanism of respiration-driven proton translocation in the inner mitochondrial membrane. Analysis of proton translocation associated with oxidation of endogenous quinol. Biochim. Biophys. Acta **387**:425-440.
208. **Pappenheimer, A. M., and E. D. Hendee.** 1949. Diphtheria toxin. V. A comparison between the diphtherial succinoxidase system and that of beef heart muscle. J. Biol. Chem. **180**:597-609.
209. **Parker, E. L., H. Sternlicht, E. T. Lode, and J. C. Rabinowitz.** 1975. The use of ^{13}C nuclear magnetic resonance of aromatic amino acid residues to determine the midpoint oxidation-reduction potential of each iron-sulfur cluster of *Clostridium acidi-urici* and *Clostridium pasteurianum*. J. Biol. Chem. **250**:2062-2072.
210. **Peck, H. D., Jr.** 1960. Evidence for oxidative phosphorylation during the reduction of sulfate with hydrogen by *Desulfovibrio desulfuricans*. J. Biol. Chem. **253**:2734-2738.
211. **Peck, H. D., T. E. Deacon, and J. T. Davidson.** 1965. Studies on adenosine 5'-phosphosulfate reductase from *Desulfovibrio desulfuricans* and *Thiobacillus thioparus*. Biochim. Biophys. Acta **96**:429-446.
212. **Peck, H. P., R. Bramlett, and D. V. DerVartanian.** 1972. On the mechanism of adenyl sulfite reductase for the sulfate reducing bacterium, *Desulfovibrio vulgaris*. Z. Naturforsch. **27b**:1084-1086.
213. **Peel, J. L.** 1960. The breakdown of pyruvate by cell-free extracts of the rumen micro-organism LC. Biochem. J. **74**:525-541.
214. **Peterson, J. A., M. Kusunose, E. Kusunose, and M. J. Coon.** 1967. Enzymatic ω-hydroxylation. J. Biol. Chem. **242**:4334-4340.
215. **Pienkos, P., V. Shah, and W. J. Brill.** 1971. Molybdenum cofactors from molybdoenzymes and *in vitro* reconstitution of nitrogenase and nitrate reductases. Proc. Natl. Acad. Sci. U.S.A. **74**:5468-5471.
216. **Prince, R. C., and P. L. Dutton.** 1976. Further studies on the Rieske iron-sulfur center in mitochondrial and photosynthetic systems: a pK on the oxidized form. FEBS Lett. **65**:117-119.
217. **Prince, R. C., J. S. Leigh, Jr., and P. L. Dutton.** 1974. An electron spin resonance characterization of *Rhodopsuedomonas capsulata*. Biochem. Soc. Trans. **2**:950-953.
218. **Prince, R. C., J. G. Lindsay, and P. L. Dutton.** 1975. The Rieske iron-sulfur center in mitochondrial and photosynthetic systems. Em/pH relationships. FEBS Lett. **51**:108-111.
219. **Que, L., Jr., R. H. Holm, and L. E. Mortenson.** 1975. Extrusion of Fe_2S_2* and Fe_4S_4* cores from the active sites of ferredoxin proteins. J. Am. Chem. Soc. **75**:463-464.
220. **Rawlings, J., V. K. Shah, J. R. Chisnell, W. J. Brill, R. Zimmerman, E. Munck, and W. H. Orme-Johnson.** 1978. Novel metal cluster in the iron-molybdenum cofactor of nitrogenase. J. Biol. Chem. **253**:1001-1004.
221. **Reddy, C. A., M. P. Bryant, and M. J. Wolin.**

1972. Ferredoxin- and nicotinamide adenine dinucleotide-dependent H_2 production from ethanol and formate in extracts of S organism isolated from "Methanobacillus omelianskii." J. Bacteriol. **110**:126–132.

222. **Reddy, C. A., M. P. Bryant, and M. J. Wolin.** 1972. Ferredoxin-dependent conversion of acetaldehyde to acetate and H_2 in extracts of S organism. J. Bacteriol. **110**:133–138.
223. **Rieske, J. S.** 1976. Composition, structure, and function of Complex III of the respiratory chain. Biochim. Biophys. Acta **456**:195–247.
224. **Rieske, J. S., D. H. MacLennan, and R. Coleman.** 1964. Isolation and properties of an iron-protein from the (reduced coenzyme Q) cytochrome *c* reductase complex of the respiratory chain. Biochem. Biophys. Res. Commun. **15**: 338–344.
225. **Rieske, J. S., W. S. Zaugg, and R. E. Hansen.** 1964. Studies on the electron transfer system. LIX. Distribution of iron and of the component giving an electron paramagnetic resonance signal at g = 1.90 in subfractions of Complex III. J. Biol. Chem. **239**:3023–3030.
226. **Riet, J. V., J. H. Ee, R. Wever, B. F. Van-Gelder, and R. J. Planta.** 1975. Characterization of the respiratory nitrate reductase of *Klebsiella aerogenes* a a molybdenum-containing iron-sulfur enzyme. Biochem. Biophys. Acta **405**:306–317.
227. **Riet, J. V., and R. J. Planta.** 1975. Purification, structure and properties of the respiratory nitrate reductase of *Klebsiella aerogenes*. Biochim. Biophys. Acta **379**:81–94.
228. **Rueger, D. C., and L. M. Siegel.** 1976. *Escherichia coli* sulfite reductase: role of sirohemesulfur complexes in the multielectron reduction process, p. 610–620. *In* T. P. Singer (ed.), Flavins and flavoproteins, Proceedings of the Fifth International Symposium. Elsevier Scientific Publishing Co., Amsterdam.
229. **Salerno, J. C., T. Ohnishi, J. Lim, and T. E. King.** 1976. Tetranuclear and binuclear iron-sulfur clusters in succinate dehydrogenase: a method of iron quantitation by formation of paramagnetic complexes. Biochem. Biophys. Res. Commun. **73**:833–840.
230. **Sauer, F. D., R. S. Bush, and I. L. Stevenson.** 1976. The separation of pyruvate-ferredoxin oxidoreductase from *Clostridium pasteurianum* into two enzymes catalyzing different reactions. Biochim. Biophys. Acta **445**:518–520.
231. **Sauer, F. D., J. D. Erfle, and S. Mahadevan.** 1975. Amino acid biosynthesis in mixed rumen cultures. Biochem. J. **150**:357–372.
232. **Scherer, P. A., and R. K. Thauer.** 1978. Purification and properties of reduced ferredoxin: CO_2 oxidoreductase from *Clostridium pasteurianum*, a molybdenum iron-sulfur protein. Eur. J. Biochem. **85**:125–135.
233. **Scherings, G., H. Haaker, and C. Veeger.** 1977. Regulation of nitrogen fixation by Fe-S protein II in *Azotobacter vinelandii*. Eur. J. Biochem. **77**:621–630.
234. **Schneider, K., and H. G. Schlegel.** 1977. Localization and stability of hydrogenase from aerobic hydrogen bacteria. Arch. Microbiol. **112**:229–238.
235. **Schneider, K., and H. G. Schlegel.** 1978. Identification and quantitative determination of the flavin component of soluble hydrogenase from *Alcaligenes eutrophus*. Biochem. Biophys. Res. Commun. **84**:564–571.
236. **Scott, R. H., and J. A. DeMoss.** 1976. Formation of the formate-nitrate electron transport pathway from inactive components in *Escherichia coli*. J. Bacteriol. **126**:478–486.
237. **Shah, V. K., and W. J. Brill.** 1977. Isolation of an iron-molybdenum cofactor from nitrogenase. Proc. Natl. Acad. Sci. U.S.A. **74**:3249–3253.
238. **Shah, V. K., L. C. Davis, and W. J. Brill.** 1975. Nitrogenase. VI. Acetylene reduction assay: dependence of nitrogen fixation estimates on component ratio and acetylene concentration. Biochim. Biophys. Acta **384**:353–359.
239. **Shanmugam, K. T., B. B. Buchanan, and D. I. Arnon.** 1972. Ferredoxins in light- and dark-grown photosynthetic cells with special reference to *Rhodospirillum rubrum*. Biochim. Biophys. Acta **256**:477–486.
240. **Shethna, Y. I., D. V. DerVartanian, and H. Beinert.** 1968. Non-heme (iron-sulfur) proteins of *Azotobacter vinelandii*. Biochem. Biophys. Res. Commun. **31**:862–868.
241. **Shethna, Y. I., N. A. Stombaugh, and R. H. Burris.** 1971. Ferredoxin from *Bacillus polymyxa*. Biochem. Biophys. Res. Commun. **42**: 1108–1116.
242. **Siegel, L. M., and P. S. Davis.** 1974. Reduced nicotinamide adenine dinucleotide phosphate-sulfite reductase of enterobacteria. IV. The *Escherichia coli* hemoflavoprotein: subunit structure and dissociation into hemoprotein and flavoprotein components. J. Biol. Chem. **249**:1587–1598.
243. **Siegel, L. M., P. S. Davis, and H. Kamin.** 1974. Reduced nicotinamide adenine dinucleotide phosphate sulfite reductase of enterobacteria. III. The *Escherichia coli* hemoflavoprotein: catalytic parameters and the sequence of electron flow. J. Biol. Chem. **249**:1572–1586.
244. **Siegel, L. M., E. J. Faeder, and H. Kamin.** 1972. Flavin interaction in NADPH-sulfite reductase. Z. Naturforsch. **27b**:1087–1089.
245. **Siegel, L. M., M. J. Murphy, and H. Kamin.** 1973. Reduced nicotinamide adenine dinucleotide phosphate-sulfite reductase of enterobacteria. J. Biol. Chem. **248**:251–264.
246. **Sieker, L. C., E. T. Adman, and L. H. Jensen.** 1972. The structure of the Fe-S complex in a bacterial ferredoxin. Nature (London) **235**:40–42.
247. **Sin, I. L.** 1975. Purification and properties of xanthine dehydrogenase from *Pseudomonas acidovorans*. Biochim. Biophys. Acta **410**:12–20.
248. **Sirevag, R., and J. G. Ormerod.** 1970. Carbon dioxide fixation in green sulfur bacteria. Bio-

chem. J. **120**:399–408.
249. **Smillie, R. M.** 1965. Isolation of two proteins with chloroplast ferredoxin activity from a blue-green alga. Biochem. Biophys. Res. Commun. **20**:621–629.
250. **Smith, B. E., and G. Lang.** 1974. Mössbauer spectroscopy of the nitrogenase protein from *Klebsiella pneumoniae*. Structural assignments and mechanistic conclusions. Biochem. J. **137**:169–180.
251. **Smith, B. E., D. J. Lowe, and R. C. Bray.** 1973. Studies by electron paramagnetic resonance on the catalytic mechanism of nitrogenase of *Klebsiella pneumoniae*. Biochem. J. **135**:331–341.
252. **Smith, L. A., S. Hill, and M. G. Yates.** 1976. Inhibition by acetylene of conventional hydrogenase in nitrogen-fixing bacteria. Nature (London) **262**:209–210.
253. **Smith, R. V., R. J. Noy, and M. C. W. Evans.** 1971. Physiological electron-donor systems to the nitrogenase of the blue-green alga *Anabaena cylindrica*. Biochim. Biophys. Acta **253**: 104–109.
254. **Smith, S. T., K. V. Rajagopalan, and P. Handler.** 1967. Purification and properties of xanthine dehydrogenase from *Micrococcus lactilyticus* J. Biol. Chem. **242**:4108–4117.
255. **Sorokin, Y. J.** 1966. Role of carbon dioxide and acetate in biosynthesis by sulfate-reducing bacteria. Nature (London) **210**:551–552.
256. **Stasny, J. T., R. C. Burns, B. D. Korant, and R. W. F. Hardy.** 1974. Electron microscopy of the Mo-Fe protein from *Azotobacter* nitrogenase. J. Cell Biol. **60**:311–316.
257. **Steenkamp, D. J., and J. Mallinson.** 1976. Trimethylamine dehydrogenase from a methylotropic bacterium. I. Isolation and steady-state kinetics. Biochim. Biophys. Acta **429**: 705–719.
258. **Steenkamp, D. J., and T. P. Singer.** 1976. On the presence of a novel covalently bound oxidation-reduction cofactor, iron and labile sulfur in trimethylamine dehydrogenase. Biochem. Biophys. Res. Commun. **71**:1289–1295.
259. **Steenkamp, D. J., T. P. Singer, and H. Beinert.** 1978. Participation of the iron-sulfur cluster and of the covalently bound coenzyme of trimethylamine dehydrogenase in catalysis. Biochem. J. **169**:361–369.
260. **Stephenson, M. P., and E. A. Dawes.** 1971. Pyruvic acid and formic acid metabolism in *Sarcina ventriculi* and the role of ferredoxin. J. Gen. Microbiol. **69**:331–343.
261. **Stombaugh, N. A., R. H. Burris, and W. H. Orme-Johnson.** 1973. Ferredoxins from *Bacillus polymyxa*. Low potential iron-sulfur proteins which appear to contain single four iron, four sulfur centers accepting a single electron on reduction. J. Biol. Chem. **248**:7951–7956.
262. **Stombaugh, N. A., R. H. Burris, and W. H. Orme-Johnson.** 1974. Oxidation-reduction potentials of ferredoxins and methyl viologen. Fed. Proc. **33**:1255.
263. **Suh, B. J., and J. M. Akagi.** 1969. Formation of thiosulfate from sulfite by *Desulfovibrio vulgaris*. J. Bacteriol. **99**:210–215.
264. **Sweeney, W. V., A. J. Bearden, and J. C. Rabinowitz.** 1974. The electron paramagnetic resonance of oxidized clostridial ferredoxins. Biochem. Biophys. Res. Commun. **59**:188–194.
265. **Sweeney, W. V., J. C. Rabinowitz, and D. C. Yoch.** 1975. High and low reduction potential 4Fe-4S* clusters in *Azotobacter vinelandii*. $(4Fe\text{-}4S^*)_2$ ferredoxin I. J. Biol. Chem. **250**: 7842–7847.
266. **Tagawa, K., and D. I. Arnon.** 1962. Ferredoxins as electron carriers in photosynthesis and in the biological production and consumption of hydrogen gas. Nature (London) **195**:537–543.
267. **Tanaka, M., M. Haniu, K. T. Yasunobu, M. C. W. Evans, and K. K. Rao.** 1974. Amino acid sequence of ferredoxin from a photosynthetic green bacterium, *Chlorobium limicola*. Biochemistry **13**:2953–2959.
268. **Tanaka, M., M. Haniu, K. T. Yasunobu, M. C. W. Evans, and K. K. Rao.** 1975. The amino acid sequence of ferredoxin II from *Chlorobium limicola*, a photosynthetic green bacterium. Biochemistry **14**:1938–1943.
269. **Tedro, S. M., T. E. Meyer, and M. D. Kamen.** 1977. Primary structure of a high potential iron-sulfur protein from a moderately halophilic denitrifying coccus. J. Biol. Chem. **252**: 7826–7833.
270. **Tel-Or, E., L. W. Luijk, and L. Packer.** 1978. Hydrogenase in N_2-fixing cyanobacteria. Arch. Biochem. Biophys. **185**:185–194.
271. **Thauer, R. K.** 1972. CO_2-reduction to formate by NADPH. The initial step in the total synthesis of acetate from CO_2 in *Clostridium thermoaceticum*. FEBS Lett. **27**:111–115.
272. **Thauer, R. K.** 1973. CO_2 reduction to formate in *Clostridium acidi-urici*. J. Bacteriol. **114**:443–444.
273. **Thauer, R. K., G. Fuchs, and K. Jungermann.** 1974. Reduced ferredoxin:CO_2 oxidoreductase from *Clostridium pasteurianum*: its role in formate metabolism. J. Bacteriol. **118**:758–760.
274. **Thauer, R. K., G. Fuchs, and K. Jungermann.** 1977. Role of iron-sulfur proteins in formate metabolism, p. 121–156. *In* W. Lovenberg (ed.), Iron-sulfur proteins, vol. 3. Academic Press Inc., New York.
275. **Thauer, R. K., E. Rupprecht, C. Ohrloff, K. Jungermann, and K. Decker.** 1971. Regulation of the reduced nicotinamide adenine dinucleotide phosphate-ferredoxin reductase system in *Clostridium kluyveri*. J. Biol. Chem. **246**:954–959.
276. **Thiele, H. H.** 1968. Sulfur metabolism in *Thiorhodaceae*. V. Enzymes of sulfur metabolism in *Thiocapsa floridana* and *Chromatium* species. Antonie van Leeuwenhoek J. Microbiol. Serol. **34**:350–356.
277. **Thorneley, R. N. F., and A. Cornish-Bowden.** 1977. Kinetics of nitrogenase of *Klebsiella pneumoniae*. Heterotrophic interactions between magnesium adenosine 5′-diphosphate and magnesium adenosine 5′-triphosphate.

Biochem. J. **165**:255–262.

278. **Thorneley, R. N. F., and R. R. Eady.** 1973. Nitrogenase of *Klebsiella pneumoniae*. Evidence for an adenosine triphosphate-induced association of the iron-sulfur protein. Biochem. J. **133**:405–408.
279. **Tortolero, M., R. Vila, and A. Paneque.** 1975. Ferredoxin-dependent nitrate reductase from *Azotobacter chroococcum*. Plant Sci. Lett. **5**: 141–145.
280. **Trotta, P. P., K. E. B. Platzer, R. H. Haschemeyer, and A. Meister.** 1974. Glutamine-binding subunit of glutamate synthase and partial reactions catalyzed by this glutamine amino transferase. Proc. Natl. Acad. Sci. U.S.A. **71**:4607–4611.
281. **Trudinger, P. A.** 1970. Carbon monoxide-reacting pigment from *Desulfotomaculum nigrificans* and its possible relevance to sulfite reduction. J. Bacteriol. **104**:158–170.
282. **Tsibris, J. C. M., R. L. Tsai, I. C. Gunsalus, W. H. Orme-Johnson, R. E. Hansen, and H. Beinert.** 1968. The number of iron atoms in the paramagnetic center (g = 1.94) of reduced putidaredoxin, nonheme iron protein. Proc. Natl. Acad. Sci. U.S.A. **59**:959–965.
283. **Tsibris, J. C. M., and R. W. Woody.** 1970. Structural studies on iron-sulfur proteins. Coord. Chem. Rev. **5**:417–458.
284. **Tso, M. W., T. Ljones, and R. H. Burris.** 1972. Purification of the nitrogenase proteins from *Clostridium pasteurianum*. Biochim. Biophys. Acta **267**:600–604.
285. **Tso, M.-Y. W.** 1974. Some porperties of the nitrogenase proteins from *Clostridium pasteurianum*. Molecular weight, subunit structure, isoelectric point and EPR spectra. Arch. Microbiol. **99**:71–80.
286. **Tso, M.-Y. W., and R. H. Burris.** 1973. The binding of ATP and ADP by nitrogenase components from *Clostridium pasteurianum*. Biochim. Biophys. Acta **309**:263–270.
287. **Tsukihara, T., K. Fukuyama, Y. Katsube, N. Tanaka, Y. Matsuura, K. Kakudo, K. Wada, and H. Matsubara.** 1978. Structure of chloroplast-type ferredoxin isolated from *Spirulina platensis* at 2.8Å resolution. Abstracts of the Sixth International Biophysics Congress, Kyoto, Japan.
288. **Uyeda, K., and J. C. Rabinowitz.** 1971. Pyruvate-ferredoxin oxidoreductase. IV. Studies on the reaction mechanism. J. Biol. Chem. **246**: 3120–3125.
289. **Uyeda, K., and J. C. Rabinowitz.** 1971. Pyruvate-ferredoxin oxidoreductase. III. Purification and properties of the enzyme. J. Biol. Chem. **246**:3111–3119.
290. **Valentine, R. C.** 1964. Bacterial ferredoxin. Bacteriol. Rev. **28**:497–515.
291. **Valentine, R. C., R. L. Jackson, and R. S. Wolfe.** 1962. Role of ferredoxin in hydrogen metabolism of *Micrococcus lactilyticus*. Biochem. Biophys. Res. Commun. **7**:453–456.
292. **Valentine, R. C., L. E. Mortenson, H. F. Mower, R. L. Jackson, and R. S. Wolfe.** 1963. Ferredoxin requirement for reduction of hydroxylamine by *Clostridium pasteurianum*. J. Biol. Chem. **238**:856–859.
293. **Van Beeumen, J., J. Deley, D. O. Hall, R. Cammack, and K. K. Rao.** 1975. A ferredoxin from *Agrobacterium tumefaciens*. FEBS Lett. **59**:146–148.
294. **Vandecasteele, J.-P., and R. H. Burris.** 1970. Purification and properties of the constituents of the nitrogenase complex from *Clostridium pasteurianum*. J. Bacteriol. **101**:794–801.
295. **Van der Westen, H. M., S. G. Mayhew, and C. Veeger.** 1978. Separation of hydrogenase from intact cells of *Desulfovibrio vulgaris*. Purification and properties. FEBS Lett. **86**:122–126.
296. **Vetter, H., Jr., and J. Knappe.** 1971. Flavodoxin and ferredoxin of *Escherichia coli*. Hoppe Seyler's Z. Physiol. Chem. **352**:433–446.
297. **Vogels, G. D., and C. Van Der Drift.** 1976. Degradation of purines and pyrimidines by microorganisms. Bacteriol. Rev. **40**:403–468.
298. **Walker, G. A., and L. E. Mortenson.** 1973. An effect of magnesium adenosine 5′-triphosphate on the structure of azoferredoxin from *Clostridium pasteurianum*. Biochem. Biophys. Res. Commun. **53**:904–909.
299. **Walker, G. A., and L. E. Mortenson.** 1974. Effect of magnesium adenosine 5′-triphosphate on the accessibility of the iron of clostridial azoferredoxin, a component of nitrogenase. Biochemistry **13**:2382–2388.
300. **Walker, M. N., and L. E. Mortenson.** 1974. Evidence for the existence of a fully reduced state of molybdoferredoxin during the functioning of nitrogenase and the order of electron transfer from reduced ferredoxin. J. Biol. Chem. **249**:6356–6358.
301. **Warringa, M. G. P., and A. Guiditta.** 1958. Studies on succinate dehydrogenase. IX. Characterization of enzyme from *Micrococcus lactilyticus*. J. Biol Chem. **230**:111–123.
302. **Watt, G. D., W. A. Bulen, A. Burns, and K. L. Hadfield.** 1975. Stoichiometry, ATP/$2e^-$ values and energy requirements for reactions catalyzed by nitrogenase from *Azotobacter vinelandii*. Biochemistry **14**:4266–4272.
303. **Wittenberger, C. L., and R. Repaske.** 1961. Studies on hydrogen oxidation in cell-free extracts of *Hydrogenomonas eutropha*. Biochim. Biophys. Acta **47**:542–552.
304. **Wong, J. Y., E. Meyer, and R. L. Switzer.** 1977. Glutamine phosphoribosyl pyrophosphate amidotransferase from *Bacillus subtilis*. J. Biol. Chem. **252**:7424–7426.
305. **Wong, P., H. J. Evans, R. Klucas, and S. Russell.** 1971. Investigations into the pathway of electron transport to the nitrogenase from nodule bacteroids, p. 525–543. *In* T. A. Lie and E. G. Mulder (ed.), Plant and soil (special volume). Biological nitrogen fixation.
306. **Yagi, T., M. Honya, and N. Tamiya.** 1968. Purification and properties of hydrogenase of different origins. Biochim. Biophys. Acta **153**: 699–705.

307. **Yagi, T., K. Kimura, H. Daidoji, F. Sakai, S. Tamura, and H. Inokuchi.** 1976. Properties of purified hydrogenase from the particulate fraction of *Desulfovibrio vulgaris* Miyazaki. J. Biochem. **79**:661-671.

308. **Yang, S.-S., L. G. Ljundahl, and J. LeGall.** 1977. A four-iron, four-sulfide ferredoxin with high thermostability from *Clostridium thermoaceticum*. J. Bacteriol. **130**:1084-1090.

309. **Yasunobu, K. T., and M. Tanaka.** 1973. The types, distribution in nature, structure-function, and evolutionary data of the iron-sulfur proteins, p. 27-130. *In* W. Lovenberg (ed.), Iron-sulfur proteins, vol 2. Academic Press Inc., New York.

310. **Yoch, D. C.** 1973. Purification and properties of two ferredoxins from the nitrogen-fixing bacterium *Bacillus polymyxa*. Arch. Biochem. Biophys. **158**:633-640.

311. **Yoch, D. C.** 1978. Nitrogen fixation and hydrogen metabolism by photosynthetic bacteria, p. 657-671. *In* R. C. Clayton and W. R. Sistrom (ed.), Photosynthetic bacteria. Plenum Press, New York.

312. **Yoch, D. C., and D. I. Arnon.** 1970. Nitrogen fixation system of photosynthetic bacteria. II. *Chromatium* nitrogenase activity linked to photochemically generated assimilatory power. Biochim. Biophys. Acta **197**:180-184.

313. **Yoch, D. C., and D. I. Arnon.** 1972. Two biologically active ferredoxins from the aerobic nitrogen-fixing bacterium, *Azotobacter vinelandii*. J. Biol. Chem. **247**:4515-4520.

314. **Yoch, D. C., and D. I. Arnon.** 1975. Comparison of two ferredoxins from *Rhodospirillum rubrum* as electron carriers for the native nitrogenase. J. Bacteriol. **121**:743-754.

315. **Yoch, D. C., D. I. Arnon, and W. V. Sweeney.** 1975. Characterization of two soluble ferredoxins as distinct from bound iron-sulfur proteins in the photosynthetic bacterium *Rhodospirillum rubrum*. J. Biol. Chem. **250**:8330-8336.

316. **Yoch, D. C., J. R. Benneman, D. I. Arnon, and R. C. Valentine.** 1970. An endogenous electron carrier for the nitrogenase system of *Rhizobium* bacteroids. Biochem. Biophys. Res. Commun. **38**:838-842.

317. **Yoch, D. C., J. R. Benemann, R. C. Valentine, and D. I. Arnon.** 1969. The electron transport system in nitrogen fixation by *Azotobacter*. II. Isolation and function of a new type of ferredoxin. Proc. Natl. Acad. Sci. U.S.A. **64**:1404-1410.

318. **Yoch, D. C., and R. P. Carithers.** 1978. Potentiometric titration of the high- and low-potential 4Fe-4S* centers of *Azotobacter vinelandii* ferredoxin I. J. Bacteriol. **136**:822-824.

319. **Yoch, D. C., R. P. Carithers, and D. I. Arnon.** 1977. Isolation and characterization of bound iron-sulfur proteins from bacterial photosynthetic membranes. I. Ferredoxins III and IV from *Rhodospirillum rubrum* chromatophores. J. Biol. Chem. **252**:7453-7460.

320. **Yoch, D. C., and R. C. Valentine.** 1972. Ferredoxins and flavodoxins of bacteria. Annu. Rev. Microbiol. **26**:139-162.

321. **Yoch, D. C., and R. C. Valentine.** 1972. Four-iron (sulfide) ferredoxin from *Bacillus polymyxa*. J. Bacteriol. **110**:1211-1213.

322. **Zubieta, J. A., R. Mason, and J. R. Postgate.** 1973. A four-iron ferredoxin from *Desulfovibrio desulfuricans*. Biochem. J. **133**:851-854.

323. **Zumft, W. B., W. C. Cretney, T. C. Huang, L. E. Mortenson, and G. Palmer.** 1972. On the structure and function of nitrogenase from *Clostridium pasteurianum*. Biochem. Biophys. Res. Commun. **48**:1525-1532.

324. **Zumft, W. G., and L. E. Mortenson.** 1975. The nitrogen fixing complex of bacteria. Biochim. Biophys. Acta **416**:1-52.

325. **Zumft, W. G., L. E. Mortenson, and G. Palmer.** 1974. Electron paramagnetic resonance studies of nitrogenase. Investigation of the redox behavior of azoferredoxin and molybdoferredoxin proteins with potentiometric and rapid-freeze techniques. Eur. J. Biochem. **46**: 525-535.

326. **Zumft, W. G., G. Palmer, and L. E. Mortenson.** 1973. Electron paramagnetic resonance studies on nitrogenase. II. Interactions of adenosine 5′ triphosphate with azoferredoxin. Biochim. Biophys. Acta **292**:413-421.